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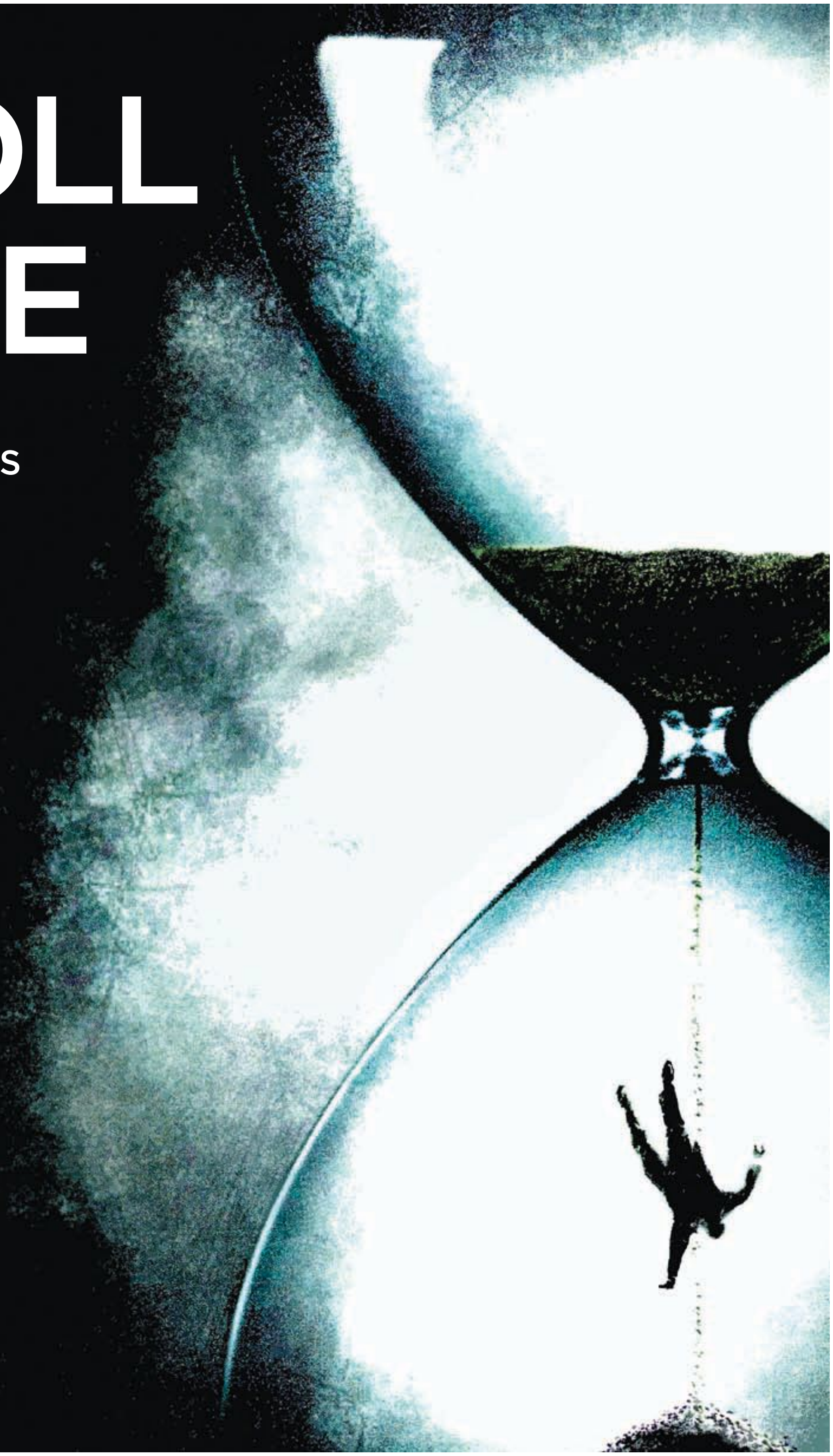

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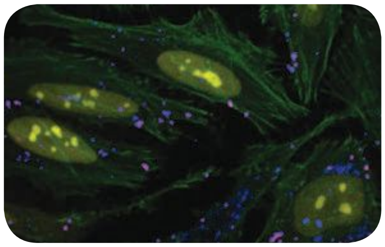

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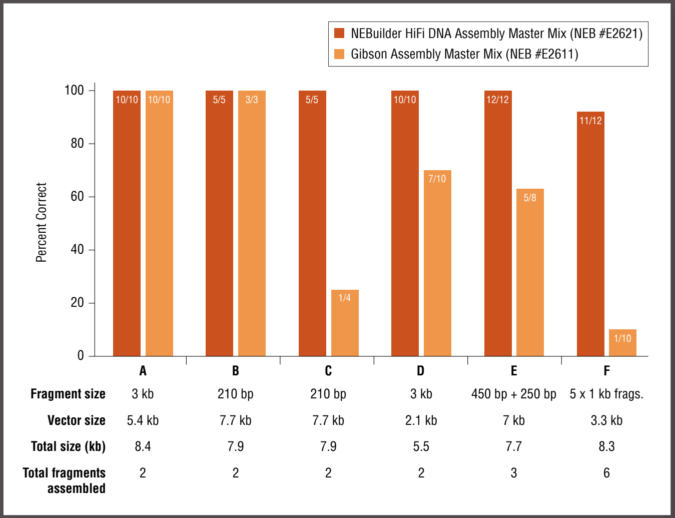

	A	B	C	D	E	F
Fragment size	3 kb	210 bp	210 bp	3 kb	450 bp + 250 bp	5 x 1 kb frags.
Vector size	5.4 kb	7.7 kb	7.7 kb	2.1 kb	7 kb	3.3 kb
Total size (kb)	8.4	7.9	7.9	5.5	7.7	8.3
Total fragments assembled	2	2	2	2	3	6

Fidelity of assembled products was compared between NEBuilder HiFI DNA Assembly Master Mix (NEB #E2621) and Gibson Assembly Master Mix (NEB #E2611). Experiments were performed using the various fragment and vector sizes, following suggested protocols. Experiments B and C vary because sequences of fragments are different. Experiments D and F were performed with fragments containing 3´-end mismatches.

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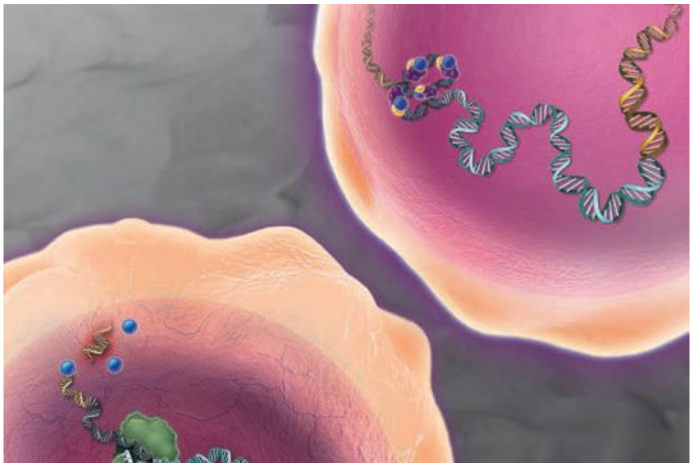

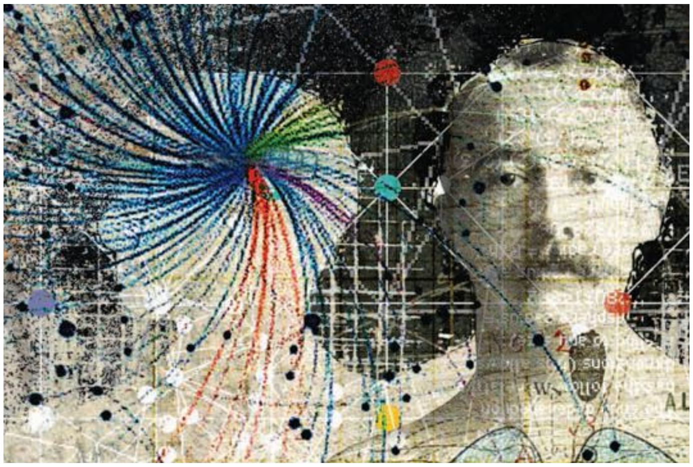

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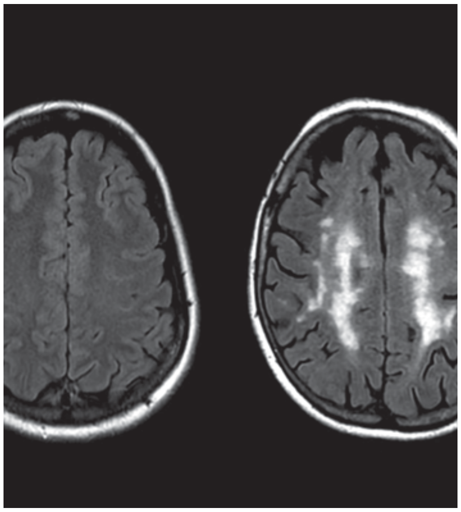

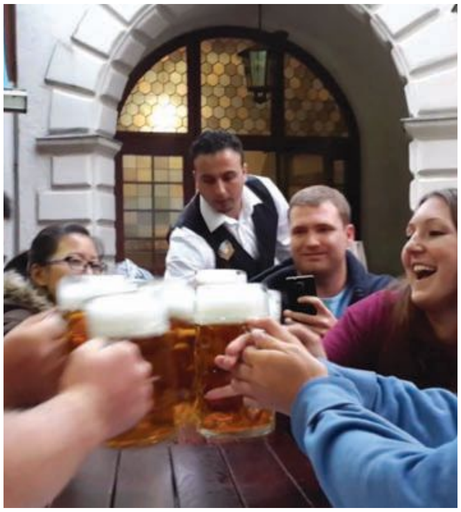

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Follow researchers at Trinity College Dublin and the University of York as they extract information about centuries-old sheep breeds by sequencing the DNA of old parchments.

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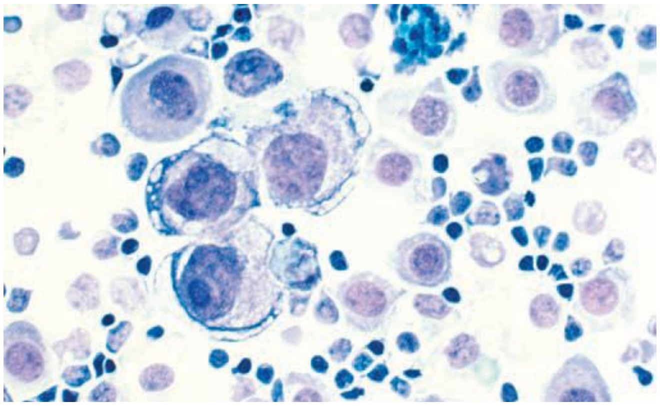

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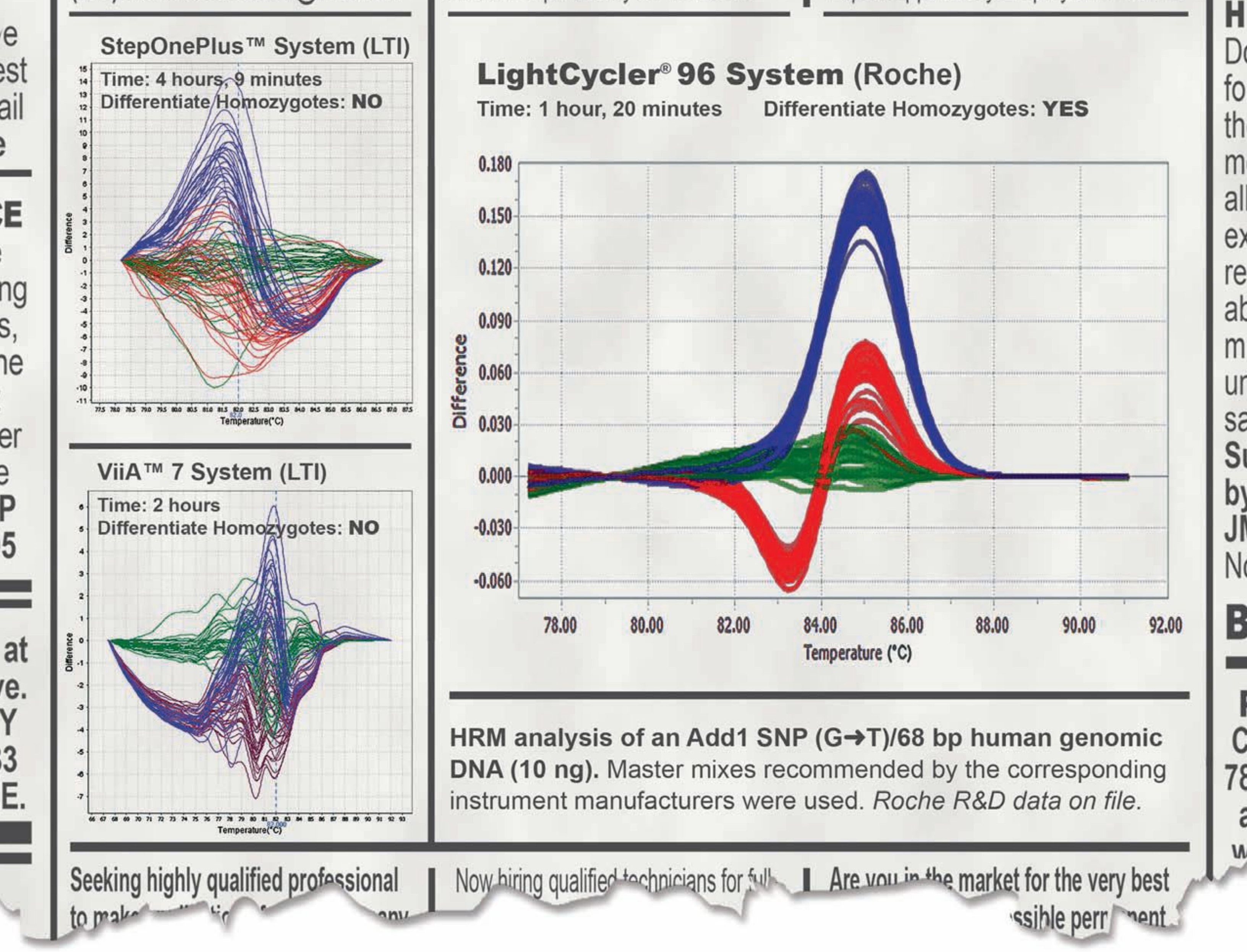

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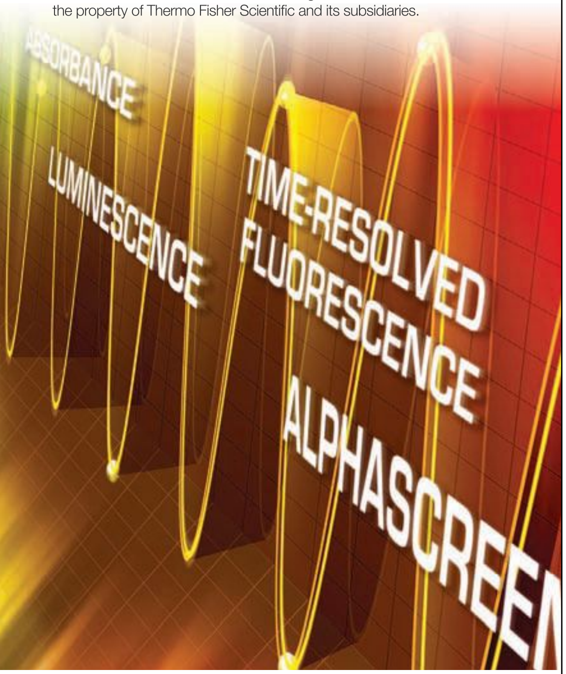

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415 Madison Avenue,
Suite 1508,
New York, NY
10017
E-mail: info@the-scientist.com

EDITORIAL

Editor-in-Chief **Mary Beth Aberlin**
marybeth.aberlin@the-scientist.com

Senior Editors **Jef Akst**
jef.akst@the-scientist.com

Bob Grant
robert.grant@the-scientist.com

Associate Editor **Kerry Grens**
kgrens@the-scientist.com

News Editor **Tracy Vence**
tvence@the-scientist.com

Contributing Editor **Alla Katsnelson**

Copy Editor **Annie Gottlieb**

Correspondents **Anna Azvolinsky**
Ruth Williams

Intern **Jenny Rood**

DESIGN AND PRODUCTION

Art Director **Lisa Modica**
lmodica@the-scientist.com

Graphic Designer **Erin Lemieux**
elemieux@the-scientist.com

MANAGEMENT AND BUSINESS

President **Bob Kafato**
bobk@labx.com

General Manager **Ken Piech**
kenp@labx.com

Managing Partner **Mario Di Ubaldi**
mariod@the-scientist.com

Publisher **Robert S. D'Angelo**
rdangelo@the-scientist.com

ADMINISTRATION

Customer Service info@the-scientist.com

Administrative Assistant **Lee Denton**
ldenton@labx.com

ADVERTISING AND MARKETING

Display Manager
New England and Mid-Atlantic **Anita Bell**
abell@the-scientist.com

Melanie Dunlop
(currently on maternity leave)

Display Manager
Western US and Canada **Ashley Haire (Munro)**
ashleyh@the-scientist.com

Engagement Manager,
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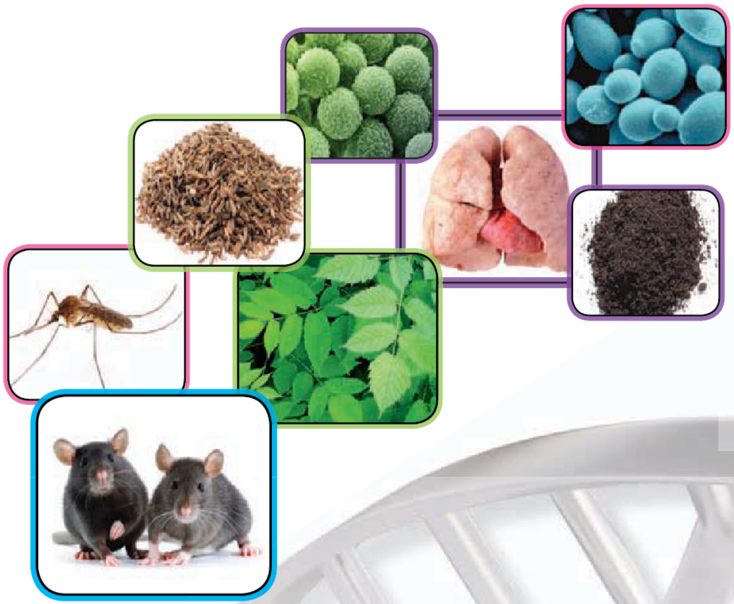

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Contributors

Morten Scheibye-Knudsen has been investigating the mysteries of mitochondria since his medical school days at the University of Copenhagen. After setting up a company that is the largest recruiter of volunteers for clinical trials in Denmark and serving as a physician both there and in Greenland, Scheibye-Knudsen moved to the National Institute on Aging in Baltimore in 2008. First as a postdoc and now as a research fellow, he has become particularly interested in the role of mitochondrial defects in diseases of accelerated aging. "Some of these acceleration disorders have a very strong neurological phenotype," he says. "And what's interesting about that phenotype is that it correlates very strongly with what's seen in mitochondrial diseases." He created an online tool, mitodb.com, which allows users to explore the role of mitochondria and their relationship to aging and the brain in several of these disorders. In the future, Scheibye-Knudsen hopes to investigate ways to positively alter metabolism in aging. "I'm very interested in pursuing the idea that metabolic interventions and treatment with key metabolites can rescue features of aging," he says. "That's something that will become very big in the coming years."

Scheibye-Knudsen explores how brain metabolism changes with age in his feature, "Nourishing the Aging Brain" (Page 46).

Michael Van Meter received his bachelor's degree from Union College in Schenectady, New York, where he majored in biochemistry. He credits much of his early interest in biology to influential mentors and undergraduate research experiences. After receiving his undergraduate degree, he enrolled in graduate school at the University of Rochester, where he studies the molecular biology of aging in the laboratory of Vera Gorbunova and Andrei Seluanov. "The more I read about aging, the more I realized how little we really know about it," Van Meter explains. "This lack of knowledge struck me as odd because aging is a universal concern." While studying a protein, SIRT6, which enhances life span in mice, Van Meter found that it regulates a damaging retrotransposon lurking in our DNA. "The idea that genomic parasites hidden within our genome might be getting loose as we get older and wreaking havoc is very exciting," he says. "It is a new direction that has the potential to change the ways in which we understand aging processes."

As a young undergraduate at Russia's St. Petersburg State University, **Vera Gorbunova** attended a scientific lecture on cellular aging. "It just made such an impression on me that I wanted to be involved in aging research," Gorbunova remembers. After finishing her bachelor's degree, however, she did her doctoral research on plant transposons in the lab of Avraham Levy at the Weizmann Institute of Science in Israel. That work provided her with insights that would shape her future interests. "Once I started working with human cells and animals, it was always in the back of my mind that transposons may be playing a role in animal aging," she says. Gorbunova completed a postdoc at Baylor College of Medicine before setting up a lab with her husband, Andrei Seluanov, at the University of Rochester. Together, they have returned to the problem that fascinated Gorbunova from the beginning. "Aging is one of the most important problems in biology and medicine right now," Gorbunova says. "Most diseases that people suffer from in developed countries are actually a consequence of aging."

Andrei Seluanov met Gorbunova while they were undergraduates in St. Petersburg. Together they dreamed about one day setting up a lab to study the biology of aging. They married before moving together to Israel to continue their education at the Weizmann Institute of Science, where Seluanov completed his doctoral work in biochemistry with Eitan Bibi before doing postdoctoral work at Baylor College of Medicine. In 2004 Seluanov and Gorbunova founded their joint laboratory at the University of Rochester. Their research focuses on cellular and molecular mechanisms of aging using traditional model systems such as human cells and mice, as well as unconventional long-lived animals such as naked mole rats, squirrels, beavers, and even whales. Gorbunova and Seluanov maintain their own colony of naked mole rats, which they compare to the more than 25 other rodent species in their collection to understand why naked mole rats enjoy such long, cancer-free lives. The longevity mechanisms revealed by this approach often contain surprises that delight Seluanov. "It's quite amazing to study these unique long-lived animals," he says. "It means you don't know what you'll get next."

Van Meter, Seluanov, and Gorbunova describe the role retrotransposons may play in aging in a feature, "Keeping Retrotransposons in Line" (Page 41).

SCHEIBYE-KNUDSEN; CARLY CHASIN; UNIVERSITY OF ROCHESTER

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Age-Old Questions

How do we age, and can we slow it down?

BY MARY BETH ABERLIN

> *To die of old age is a death rare, extraordinary, and singular, and therefore so much less natural than the others. 'Tis the last and extremest sort of dying, and the more remote the less to be hoped for. It is indeed the boundary of life, beyond which we are not to pass: which the law of nature has pitched for a limit not to be exceeded.*
>
> —Michel de Montaigne, *Essays*, Book I, Chapter 57

This centuries-old description by the French essayist perfectly captures just how peculiar it is to arrive at the end of one's life having escaped death by infection or diseases such as cancer, heart disease, and dementia, for which age is the biggest risk factor. Although Montaigne himself reached what was at the time considered old age, succumbing to a bacterial infection in 1592, when he was 59 years old, he certainly did achieve literary immortality.

This issue of *The Scientist* is about aging, and the focus is not on the diseases that are hallmarks of time's passage, but on why cells have a limited life span and what fails on the cellular and molecular level as the years go by. It's a complicated picture, with lots of missing pieces. The field of cellular biogerontology was kick-started by the observation, published in 1961, that normal cells in culture stopped dividing after 50 or so doublings—the well-known Hayflick limit. In "Of Cells and Limits," on page 54, you can meet Leonard Hayflick and learn of his dogma-defying discovery in a profile of the unretired (and unretiring) octogenarian's contributions to the understanding of cellular aging.

A staff-written feature describes some of the knowns and unknowns of "How We Age" (page 33) at the level of the genome, cell, and whole organism. "Wrangling Retrotransposons" (page 41) lays out a possible connection between aging and a mobile genetic element called long interspersed element-1 (LINE-1 or L1), as it roams around the genome inserting itself in damaging places. Authors Michael Van Meter, Andrei Seluanov, and Vera Gorbunova write that "because genomic instability and DNA damage are key features of aging, it is easy to envision how L1s may contribute to the molecular etiology of the aging process." In "Nourishing the Aging Brain" (page 46), Morten Scheibye-Knudsen takes a hard look at the brain's nutritional requirements, what is known about neurological brain aging, and the suggestion that caloric restriction could increase human life span and ameliorate some of the effects of aging-related diseases.

ANDRZEJ KRAUZE

The Literature section is devoted to three recent papers that raise interesting possibilities for aging research, albeit using different model organisms: human telomeres' changing regulation of gene expression as a function of their shortening; roundworms' upregulation of collagen production and the extension of life span; and the interaction between growth hormone signaling and a life span–extending dietary supplementation in mice.

Whether human life span can be extended beyond the limit of about 120 years remains to be seen. Heaven knows longevity is a wish as old as life itself, and now serious science is shouldering aside the quacks, committing research and funding to probe the real possibility of lengthening our time on the stage. Ankita Bansal and Heidi Tissenbaum ("Quantity or Quality" on page 30) sound a cautionary note that increased life span must be accompanied by an increase in health span, lest we merely spend more years in decrepitude; and Matthias Ziehm urges the sharing of data to help further longevity research (page 28).

You won't be that much older by the time you finish reading this issue, but I hope you take away some provocative new clues to how we all—eventually—age. ■

MBA

Editor-in-Chief
eic@the-scientist.com

Speaking of Science

Quam continuis et quantis longa senectus/Plena malis!
How incessant and great are the ills with which a prolonged old age is replete.

—**Juvenal**, late 1st-century Roman poet

Ultimately, all medical research is aimed at prolonging life—but we must strive to prolong *healthy* life. Therefore, our challenge remains defining health and the ability to test and quantify healthy living.

—**Heidi Tissenbaum**, University of Massachusetts Medical School biologist who recently published a *PNAS* study reporting that all long-lived *C. elegans* mutants studied by aging researchers spent a higher percentage of their extended life span in frail, rather than healthy, states (January 26)

These results suggest that only a third of the variation in cancer risk among tissues is attributable to environmental factors or inherited predispositions. The majority is due to "bad luck," that is, random mutations arising during DNA replication in normal, noncancerous stem cells.

—Johns Hopkins University biologists, **Cristian Tomasetti and Bert Vogelstein**, writing in their recently published *Science* paper, in which they claim that two-thirds of the variation in cancer risk among people can be chalked up to chance (January 2)

We find that many highly educated, well-informed, religious individuals accept scientific theories about topics such as geology, radioactivity, planetary motion, genetics, and probability while rejecting mainstream scientific accounts of evolution and the big bang.

—**Timothy O'Brien**, sociologist at the University of Evansville and the lead author of a February *American Sociological Review* study, which found that roughly one in five Americans are deeply religious, know a lot about science, and support many practical uses of science and technology in everyday life, but reject scientific explanations of creation and evolution (January 29)

Twenty-first century businesses will rely on American science, technology, research and development. I want the country that eliminated polio and mapped the human genome to lead a new era of medicine—one that delivers the right treatment at the right time.

—**President Barack Obama**, in his State of the Union Address before the US Congress (January 20)

Climate is changing, and climate has always changed and always will. There's archaeological evidence of that. There's biblical evidence of that. There's historic evidence of that. . . . The hoax is that there are some people who are so arrogant to think that they are so powerful, they can change climate. Man can't change climate.

—**Jim Inhofe** (R-OK), new chairman of the Senate Committee on Environment and Public Works, restating his position on climate change (January 22)

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Notebook

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PUZZLE MASTERS: The network of researchers that participate in DREAM-sponsored challenges provide pieces of the solution to important biomedical problems. Problems range from very basic science questions such as the inference of biological networks, to translational problems, such as models for disease prognosis and treatment response. To date DREAM has curated 32 open challenges.

DANIEL MARBACH AND VALDO PEIXOTO

Beneficial Stats

Statistician Liuxia Wang is in the business of prediction. At Sentrana Inc., a Washington, D.C.-based sales and marketing company, she uses data on consumers' spending behaviors to forecast future trends in the food-service industry. But she never imagined her analytical expertise could be put to use helping patients suffering from amyotrophic lateral sclerosis (ALS), the fatal neurodegenerative disease commonly known as Lou Gehrig's disease.

"At the end of the day, data is data, whether you're using it to find the optimal price for certain products or estimating a disease's course," says Wang. "But it's nice to be able to use predictive analytics to benefit patients."

Working under the team name "Sentrana," Wang and her colleague Guang "Eric" Li, a quantitative modeler, beat a global community of experts in statistics, machine learning, and computational biology to win the ALS Prediction Prize. Hosted by the ALS-focused Prize4Life and the cross-institutional collaboration DREAM (Dialogue for Reverse Engineering Assessments and Methods), the crowdsourced event challenged number crunchers to develop a computer algorithm to predict ALS disease progression using clinical-trial data from the Pooled Resource Open-Access ALS Clinical Trials (PRO-ACT) database.

Team Sentrana shared first prize with a separate team comprising Lester Mackey, a Stanford University postdoc, and Lilly Fang, a lawyer. The two teams each won $20,000, and a runner-up—University of Zurich statistician Torsten Hothorn—received $10,000.

Overall, the two top algorithms predicted disease progression better than both a baseline model and a model developed by a dozen ALS clinicians—improvements that challenge organizers estimate could allow a drug sponsor to reduce the size of a Phase 3 clinical trial by at least 20 percent, saving as much as $6 million.

Each year, about 5,000 people in the U.S. are diagnosed with ALS, with most succumbing to the disease within three to five years. But some people with ALS, such as baseball great Lou Gehrig, live for less than three years, while others, such as physicist Stephen Hawking, live for decades.

"When I see patients, the number one question I get is, 'What is going to happen to me?' Unfortunately, we really aren't able to predict that," says Stephen Kolb, Director of the ALS/Motor Neuron Disease Clinic at Ohio State University Wexner Medical Center.

> **At the end of the day, data is data, whether you're using it to find the optimal price for certain products or estimating a disease's course.**
>
> —Liuxia Wang, Sentrana Inc.

The disease's extraordinary variability doesn't just leave patients in the dark, says Neta Zach, Prize4Life's chief scientific officer, it also presents "massive challenges" in developing new treatments for the disease, as drug trials need large cohorts of ALS patients to detect significant therapeutic effects. If the ALS Prediction Challenge can inspire more accurate methods of estimating disease progression, clinical trial design and execution could be improved, increasing the likelihood of bringing new treatments to market.

"This knowledge will also help us have more-meaningful discussions with patients and their families about disease management and end-of-life care," Kolb says.

As is often the case in computational challenges, the majority of the ALS Prediction Prize competitors had zero previous experience with the disease at hand.

"My client's son had recently been diagnosed with ALS, and she asked me if the analytics we used to develop commercial solutions could be used to make progress in the disease," Wang says. "I thought that I should try to do something to help."

IBM researcher Gustavo Stolovitzky, who founded and heads up DREAM and has run more than 30 DREAM challenges, says the ALS Prediction Prize's ability to attract a diverse group is one of its greatest strengths. "The researchers who have the best answer to a given scientific problem may not be the same researchers that generated the data or formulated the scientific questions," he says. "Why not ask everybody who is ready to roll up their sleeves, even if they come from other fields?"

More than 1,000 solvers competed for the ALS Prediction Prize. They were given access to clinical data from 1,822 ALS patients in the PRO-ACT database collected during the three months following their diagnoses and asked to develop and train algorithms that predicted the condition of each patient nine months later according to the ALSFRS, a standard functional scale that measures patients' abilities to move, care for themselves, speak, and breathe. A total of 37 algorithms were submitted.

InnoCentive, an organization that hosts crowdsourced competitions, evaluated the algorithms by comparing the predicted with the actual state of each patient. The algorithms were then assessed using a third, fully blinded and previously unseen validation set. The winning models were selected according to performance on this validation set.

The Sentrana team's algorithm showed that the speed of decline or stability of face-related ALSFRS scores, which include tests of speech and swallowing, predicted overall disease trajectory. The algorithm also showed that a decline in breathing-related scores signaled the end stage of the disease. The Stanford team's algorithm found that the elapsed time from disease onset to when patients entered clinical trials best predicted the nine-month trajectory of ALS, and that past rate of decline on the ALSFRS, fluctuations in body weight, and speaking ability were also highly predictive of disease progression.

Prize4Life CSO Zach says the two winning algorithms, which were recently published in *Nature Biotechnology* (33:51-57, 2015), "were essentially identical in predictiveness."

The challenge also identified several potential nonstandard predictors of disease progression—uric acid, creatinine, and, surprisingly, blood pressure—that may shed light on ALS pathobiology. These findings compelled challenge organizers to host a second ALS Prize4Life challenge—the ALS Stratification Challenge—that will ask solvers to develop computer algorithms that classify 9,000 ALS patients according to disease characteristics.

The ALS Stratification Challenge is hosted by Prize4Life, DREAM, and Sage Bionetworks, which merged its open-science efforts with DREAM in 2013. The new challenge will begin registration this May and launch in June. In addition to crowdsourcing a community of solvers, the collaborators are crowdfunding donations for the prizes that will be awarded to winners of the competition. **—Aimee Swartz**

A Deathly Pallor

Evolutionary biologist Kenneth Fedorka wasn't aiming to explore how cricket populations changed color in response to a warmer climate. But when the University of Central Florida researcher reared groups of crickets in his lab at higher temperatures, a change in one phenotypic trait stood out above the rest: they were a lighter shade of brown than crickets reared at cooler temperatures.

Fedorka had originally set out to understand how climate change could alter important milestones for crickets, such as whether their eggs develop or lie dormant. "It was only through happenstance that we noticed a difference in cuticle color in young adult crickets," Fedorka says. "That got the gears turning about what other effects lighter cuticles could have."

Crickets are far from the first insects that biologists have shown get lighter in warmer environments. Reports dating back to the 1930s described how beetles, flies, and butterflies living closer to the equator or at lower altitudes tended to have less melanin in their cuticles or

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wings. Recent studies have found a correlation between global warming in the last several decades and lighter beetles in the Netherlands as well as paler dragonflies across Europe (*Heredity*, 107:574-78, 2011; *Nat Commun*, 5:3874, 2014).

It's pretty clear what insects gain by adjusting their melanin in different environments. In cold, dry climates, the pigment absorbs heat and keeps them from drying out, whereas in tropical areas it could put them at risk of overheating.

But Fedorka wondered whether making less melanin could actually harm insects by weakening their defenses. "I said, 'Wait a minute, we know that melanin is part of the immune system, so the thermal environment could be shaping the immune system in maladaptive ways,'" he recalls.

Although melanin itself has antimicrobial activity and can encapsulate invading organisms in both the cuticle and within the body of insects, the real immune benefit probably comes from the biochemical production of the pigment. In response to infection or other stimuli, immune cells release an enzyme called phenoloxidase (PO), which triggers a molecular cascade that results both in compounds that have potent antimicrobial activity and in melanin, which darkens and strengthens the cuticle.

"Phenoloxidase is a workhorse that responds to viruses, bacteria, and multicellular organisms," says Shelley Adamo, a professor of psychology and neuroscience at Dalhousie University in Nova Scotia. But there are many other immune mechanisms in insects, Adamo adds, including phagocytosis and antimicrobial peptides, which may also be important—or even more important—than the PO pathway for some pathogens.

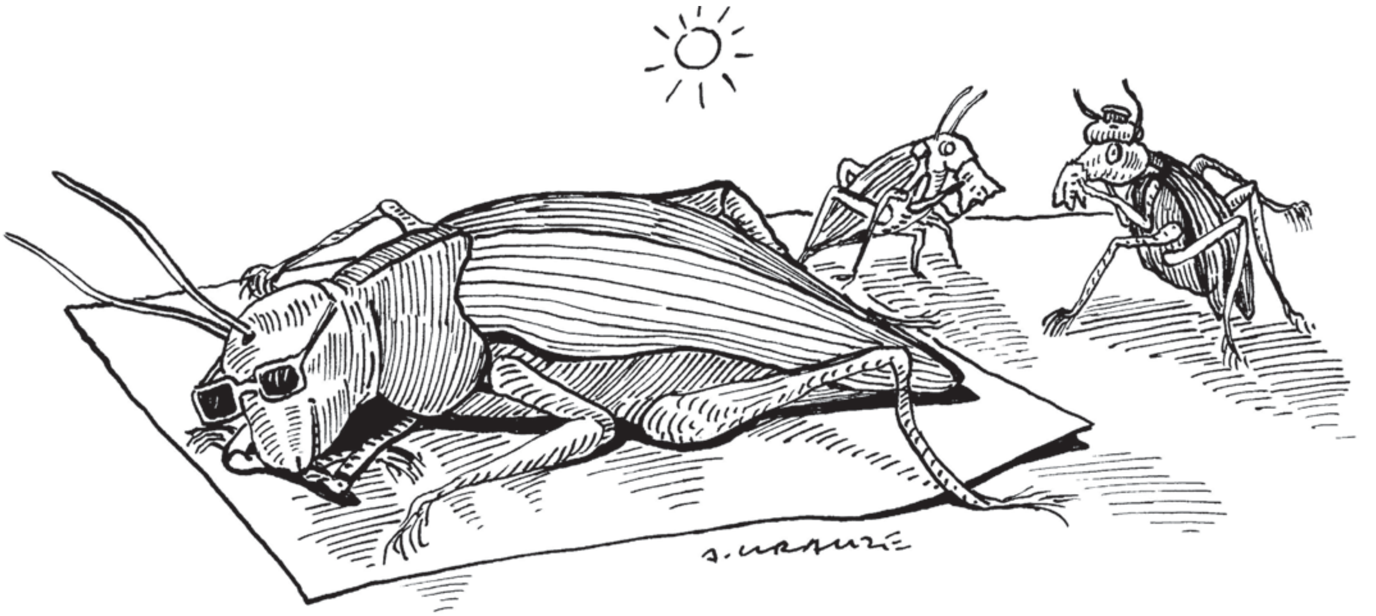
If a warmer environment means less melanin, Fedorka had a hunch it could also mean a less active PO pathway. He incubated crickets during their nymphal stage, between the time they hatch and when they become adults, at typical summer and fall daytime temperatures (28.5 °C and 23.5 °C, respectively). During this developmental stage, the expression level of genes involved in melanizing the cuticle is established. As adults, the crickets reared in summer temperatures had lighter cuticles, as predicted, and also lower levels of PO activity in their blood. Most importantly, they paid an immune price by being more likely to die after infection with a common cricket bacterium, *Serratia marcescens* (*J Exp Biol*, 216:4005-10, 2013).

This study suggests that cold-reared crickets "heavily invested in melanin-producing infrastructure, so once they get the signal, 'We are under attack,' they already have a much larger standing army to keep the invader from getting out of hand," Fedorka says. Although insects reared in the warm environment could probably ramp up PO activity, they might not be able to do so quickly enough to respond to invading pathogens, he adds.

To test whether temperature could affect the immune function of other insects, Fedorka's group did a similar study in *Drosophila*. Flies reared at 28.5 °C were paler than those reared at 21.5 °C, and their immune response also paled in comparison. *Pseudomonas aeruginosa* infection killed 75 percent more warm temperature–reared flies (*J Exp Biol*, 217:3664-69, 2014).

The similar finding in the evolutionarily distant organisms raises the possibility that hotter weather could make many other insect species less able to fend off pathogens. Mosquitoes and honeybees, both of which have melanin and the PO pathway as part of their immune system repertoire, could become even better vectors of disease or less efficient pollinators, respectively. "If this is as widespread as we hypothesize it is, then we are talking about disruption of agricultural networks and human pathogen interactions," Fedorka says.

One of Fedorka's long-term goals is to rear mosquitoes in the lab for 25 generations, incubating each generation in a slightly warmer environment to mimic the postulated climb in average air temperature in Florida between now and 2100. He predicts that they will get lighter and have weaker immune systems—and,



ANDRZEJ KRAUZE

> **If this is as widespread as we hypothesize it is, then we are talking about disruption of agricultural networks and human pathogen interactions.**
>
> —Kenneth Fedorka,
> University of Central Florida

importantly, that such changes would allow the malaria parasite *Plasmodium* to replicate more efficiently.

According to Kristin Michel, an entomologist at Kansas State University, it will also be interesting to see how warmer temperatures affect the ability of mosquitoes to control bacterial and fungal infections. (Melanin is probably more important for combating these pathogens than it is for fighting protozoan parasites, she points out.) "[This] experiment will give an idea of how mosquitoes respond to infection, and if it kills them off, it could reverse some of the range expansion predicted for climate change," Michel says.

However, before mosquitoes die off, there would be enormous selective pressure for them to compensate for the loss of PO activity, possibly by ramping up antimicrobial peptides or other unrelated immune activities, Michel says.

It is unclear at this point what the effect of the weakened PO activity in Fedorka's crickets and flies could mean for a wide range of pathogens—and for insects in the wild. "When thinking about how temperature is going to affect a species of insect, you have to think about who its pathogens are and how they respond to increases in temperature," Adamo says. For now, biologists generally expect that many pathogens will become more prevalent at higher temperatures, requiring insects to mount better, not weaker, immune responses.

—Carina Storrs

Slip Me Some Skin

The Borthwick Institute for Archives, housed at the University of York in the U.K., boasts thousands of church documents, some of which date back to before 1300, as well as troves of medical illustrations, architectural drawings, and records of the British-Caribbean slave trade. But recently, a team of biologists began digging into the Borthwick's records in search of a different sort of information: genetic material that is yielding insights into the selective breeding of farm animals.

Rather than rely solely on scarce, weather-beaten bones to reconstruct the animals' genetic histories, the researchers decided to extract DNA from pieces of 17th- and 18th-century parchment. Produced from the skins of sheep, goats, and cattle, parchment was commonly used as a writing substrate until paper came into wide use in the 19th century.

Beginning with the Dead Sea Scrolls in 1996, a handful of researchers have used polymerase chain reaction (PCR) to amplify bits of high-copy mitochondrial DNA from parchment, but these investigations have yielded little information beyond species identification. PCR favors longer stretches of DNA, which often come from contaminants, and can also produce chimeric sequences when, for example, a sheep DNA fragment is incompletely amplified, and a bit of goat DNA attaches to the amplicon in its place.

Matthew Teasdale and Daniel Bradley of Trinity College Dublin and their colleagues were the first to apply next-generation sequencing to DNA from parchment (*Phil Trans R Soc B*, 370:20130379, 2014).

"The key parameter with historical DNA is simply how much of your target species DNA is there," says Bradley. "And parchments have a very high percentage of endogenous DNA compared to contemporaneous bone samples." Parchment documents also offer the advantage of more precise dating.

To test the feasibility of high-throughput sequencing for analyzing animal genomes from parchments, Teasdale and Bradley teamed up with bioarchaeologists at the University of York and Borthwick archivist Christopher Webb.

"At the beginning of the process, we didn't know how much parchment would be needed in order to get a decent DNA record," says Webb. He advised the researchers to start with "conservation fragments—in other words, bits of parchment that we've taken off records that are of no significance in themselves," he explains. "They were only taken off because the records were themselves damaged and needed to be conserved and repaired."

The team took 25 mm^2 pieces from a pair of damaged parchments in the Borthwick archives: a 17th-century title deed and the cover of an 18th-century parish register book. Prior work on English parchments suggested these were made of sheepskin, which the team confirmed using mass spectrometry analysis of the protein collagen—parchment's main component. The manuscript dates spanned a period of major sheep-breeding advances in England, when selective mating efforts greatly enhanced the quality of wool.

Both parchments yielded complete mitochondrial genomes, as well as nearly 10 percent of the sheep nuclear genome—an improvement over the 0.1 to 5 percent coverage obtained from bone samples. Contamination rates from both modern and historical human DNA were around 5 percent.

"Next-generation sequencing is such a good technique" for ancient DNA studies, says Bradley. Because "you're analyzing everything," he says, contamination "just seems to melt away . . . we can analyze our way through it."

The researchers compared single-nucleotide polymorphisms (SNPs) from the parchment DNA to those found in modern sheep breeds. The SNPs from the 17th-century parchment matched the genotypes of heritage black-faced sheep from northern Britain, while the 18th-century sample was similar to sheep varieties from southern regions, which were hotbeds of sheep-breeding innovation.

Encouraged by this glimpse into sheep history gleaned from just two parchments, Bradley and his colleagues are now analyzing more Borthwick documents to get a fuller picture of the genomic changes that occurred during selective breeding. They also expect Irish archives to tell the history of cattle changes, and goat-based parchments to reveal breeding trends in southern Europe.

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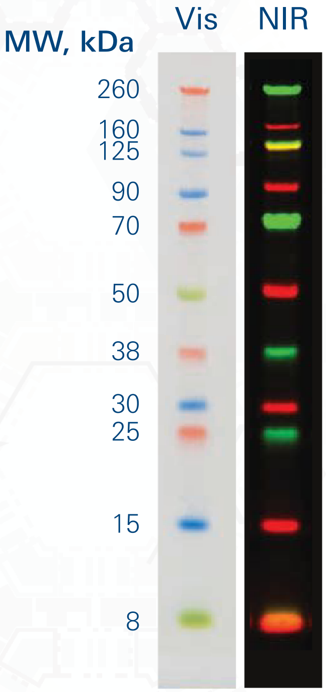

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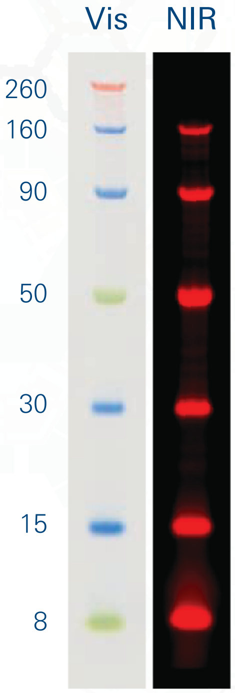

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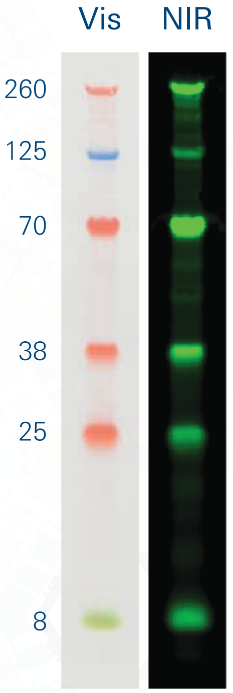

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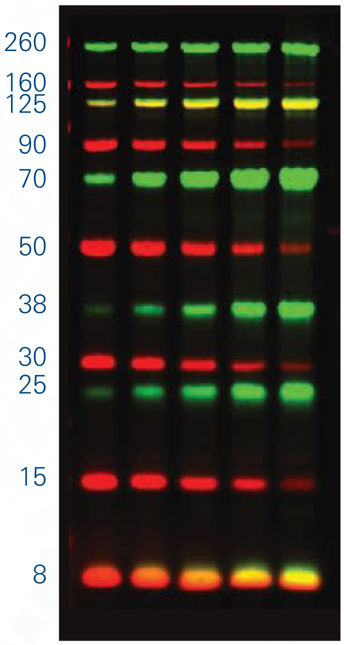

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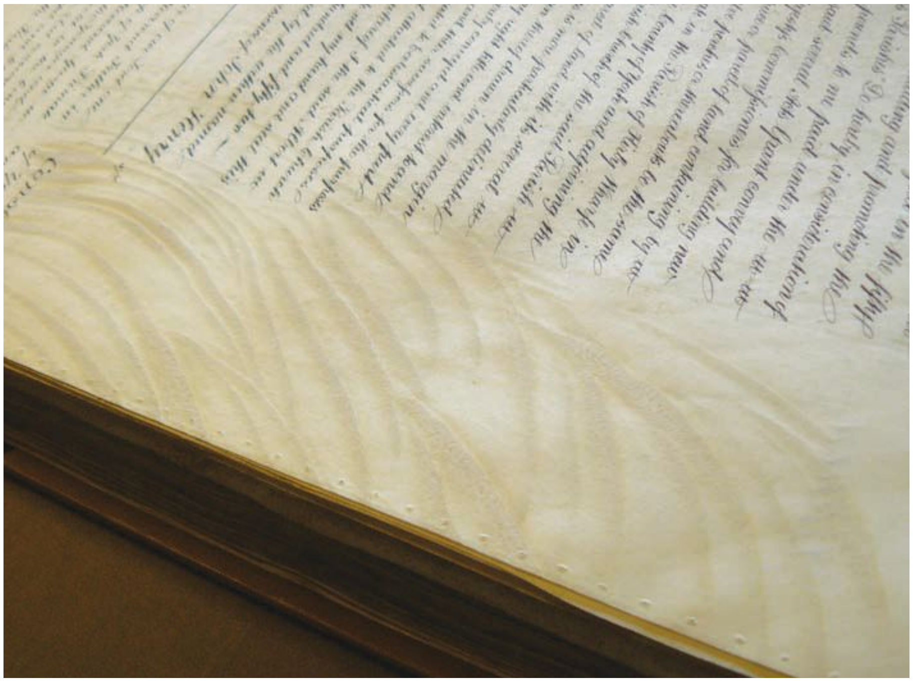

SKIN IN THE GAME: Parchment manufacture marks in a church register from 1853–1858 (left), and a sewn repair in an even older manuscript from 1306–1311 (below)

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"The genome change in sheep is a big deal in this period," says Bradley. "A lot of England's wealth is built around the wool trade, and in terms of breeding, this is where it's happening. So [this work] opens up a tantalizing window as to what a more concentrated analysis might reveal."

The group's preliminary findings differ from the last published analysis of parchment DNA. In a PCR-based study, Michael Campana and his colleagues at the University of Cambridge amplified nuclear and mitochondrial DNA fragments of multiple species from each of a dozen 18th- and 19-century English parchments (*J Archaeol Sci*, 37:1317-25, 2010).

"It looked like you were PCRing this horrible soup rather than getting good data from one individual," says Bradley. The intermingled sequences suggested complex parchment manufacturing processes, making DNA analysis potentially useful for understanding parchment sources, but not necessarily for tracking the history of animal husbandry.

Campana thinks the next-generation sequencing approach is promising, but cautions that many parchments may contain mixtures of species. "Parchment manufacture is complicated and extremely variable," says Campana, who is now investigating the history of infectious diseases at the Smithsonian Institution in Washington, D.C. "There's no reason to believe that one set of parchments represents all sets of parchments."

Besides reconstructing animal breeding histories, Webb hopes DNA analyses will help to answer another set of questions. Historians still know very little about the intricacies of parchment manufacturing and how raw-material sourcing differed by region and time period, he says. "One of the most interesting questions for me, as an archivist looking at the archives of long-lived institutions, is: Where did they get their parchment from?"

"We have absolutely no idea," says Webb, whether the Diocese of York sourced its parchments locally, from London, or from all over England. "Depending on what the answer is, that tells us a lot about medieval economy and trade." **—Molly Sharlach**

Falling Out of the Family Tree

Why are humans so attracted to alcohol, and why do so many struggle with its abuse? These are questions that science still can't answer. The "drunken monkey" hypothesis proposed by University of California, Berkeley, biologist Robert Dudley posits that, unlike our attraction to other addictive drugs, our use and sometimes dependence on alcohol stems from the millions of years our ancient primate ancestors spent consuming ripe, fermented fruits. Perhaps these predecessors evolved some kind of neurological mechanism that drove them to seek out and consume sugar-rich, ethanol-containing foods, which were likely in short supply millions of years ago. But now that there's a liquor store on every other corner, that drive to seek out alcohol has turned against us. (See "Drunks and Monkeys," *The Scientist*, June 2014.)

"There's all these anecdotal stories of . . . a monkey eating rotten fruit and getting tipsy," says Matthew Carrigan of Santa Fe College in Gainesville, Florida. "But what hasn't been done is to actually apply a real scientific test of this theory." So, during his time at the nearby Foundation for Applied Molecular Evolution, which emphasizes a paleogenetic approach to understanding human health problems, Carrigan decided to explore the evolutionary history of our ability to metabolize booze.

Specifically, Carrigan and his colleagues examined sequence changes in class IV alcohol dehydrogenases (ADH4) over the past 70 million years of primate evolution. ADH4, an enzyme found in the mouth, esophagus, and stomach of primates, is the first ethanol-digesting molecule that an alcoholic drink encounters when it enters the body. Comparing the ADH4 sequences of 28 different mammals, including 17 primates, the researchers reconstructed nine ancestral versions of the protein, which they then synthesized and analyzed for their ability to digest alcohol. While the more ancient proteins were able to oxidize some alcohols that are found in plant leaves, they were largely inactive against ethanol. That is, until about 10 million years ago, when a single amino acid replacement rendered ADH4 40-fold better at the job, allowing the animals encoding this enzyme to consume more ethanol without feeling its intoxicating effects.

This was after orangutans had branched off from the human lineage, but before chimps and gorillas had, so the latter species share this improved ethanol-digesting ability with humans. But what's even more interesting about the timing, says Carrigan, is that 10 million years ago was around the time that researchers believe this branch of arboreal primates started spending more of their lives on the ground. "The more I thought about it, the more it became interesting and potentially really important," he says. "Fruit ferments as it gets old, and where do you find old fruit? Well, older fruit is more often found on the ground."

More than 15 years ago, New Mexico State University paleoanthropologist Brenda Benefit uncovered fossil evidence that fruit-eating monkeys in Kenya 15 million years ago were adapted to walk and run on the forest floor, leading her to speculate that diet could have been a driving factor in the shift toward terrestrial living. "Back in the '90s, [we] thought that one of things that would have pulled these [primates] down to the ground would have been to forage for fallen fruit," she says. "For me, that's the aspect [of the new study] that was so fulfilling as a scientist—to have [Carrigan's] project confirm that from a completely independent avenue."

Not everyone is convinced, however. Although the timing is suggestive, "it's not at all clear that the appearance of [the mutated] ADH4 was an adaptation [to eating fermented fruit]," Doug Levey of the National Science Foundation writes in an e-mail to *The Scientist*. "[R]otten fruits have no nutritional benefits over ripe fruits and . . . the diets of most primates are quite general," he says. "[They] can presumably be selective about which fruits (rotten vs. ripe) they consume."

"My guess is that our ancestors and modern-day primates will behave a lot like you in the situation: if you eat two fruits, one's fermented and one's not, you're probably not going to eat the rotten one," agrees Carrigan, who now hopes to conduct field studies to examine primates' diets and measure the ethanol content of the fruit they're eating. But, he adds, "if you're really hungry, and there's only a rotten one available, you might eat it."

Moreover, there may be something to be said for the mind-altering effect of alcohol that is known to be a motivating factor for its consumption in human societies. "[T]he authors have presented evidence for an intriguing association between transition to a terrestrial life and appearance of the ability to metabolize ethanol," says Levey. "That association may be an evolutionary precursor to humans' attraction to alcohol."

"Alcohol is so central to human culture," adds Patrick McGovern, scientific director of the Biomolecular Archaeology Project for Cuisine, Fermented Beverages, and Health at the University of Pennsylvania Museum in Philadelphia and author of *Uncorking the Past: The Quest for Wine, Beer, and Other Alcoholic Beverages*. "It still is a prime part of human existence." **—Jef Akst**

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Sharing Longevity Data

Aging research would greatly benefit from consistently making annotated life span data available.

BY MATTHIAS ZIEHM

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Since the early days of protein crystallography, scientists have shared their research data through the Protein Data Bank, allowing new structures to be determined more easily and paving the way for structure-based drug development. Similarly, the free and open sharing of the human genome sequences has been essential for many achievements in human biology and health research. Sharing well-annotated life span data could provide a similar boost to aging research.

Recent decades have seen an explosion of knowledge on aging at the molecular level, especially in model organisms such as *Caenorhabditis elegans*, *Drosophila*, and mice. Researchers have identified various interventions—such as gene knock-outs, drug administration, dietary restriction, or altered environmental conditions—that can extend the life span of these animals in the lab. Importantly, while such manipulations can cause negative side effects such as reductions in size, fertility, or immune function, many interventions keep animals youthful—more agile, for example, or with better memory—for longer, rather than simply extending the moribund period at the end of life. (See "Quantity or Quality?" on page 30.) Moreover, research has revealed that the effects of these interventions are often evolutionarily conserved between very different organisms, suggesting they might apply to humans as well. Despite these achievements and the quickly increasing number of studies, the results still remain sparsely connected pieces of a large puzzle whose picture and dimensions are not yet clear. Sharing life span data could help connect those pieces.

A key approach to identifying factors that influence aging, and interventions that counteract aging, is to compare the life spans of groups of animals that are observed from birth or the start of adulthood until their natural death. Since predators, food competition, and other external hazards are excluded under lab conditions, in theory the life spans of the animals are determined by aging alone. Recording natural life span has the advantage over observing other signs of aging, such as greying of hair or wrinkling of skin, in that death is universally and unambiguously defined. However, usually only processed results of the analysis or a graphical representation thereof are published, while the original data are not made available.

Making such numerical longevity data available would allow other scientists to redo and check analyses, as well as apply new or different analysis methods. Moreover, having a large collection of data annotated with experimental conditions would enable researchers to jointly analyze data measured under similar conditions, or to apply statistical methods to combine dissimilar data sets while accounting for differences between them. In this way, insights beyond the scope of individual studies can be gained, including the characterization of variation between experiments, seasonal fluctuations in life spans of animals in controlled lab environments, and how life spans vary over years of experiments or among different locations. In addition, such a large set of life span data would allow us to establish the typical survival distribution under commonly used control conditions. This concept of grouping previous observations under control conditions, called the historical control, has been widely used in toxicity studies and is required by regulatory bodies to ensure that control animals exhibit normal survival.

In 2013, as a first step toward the widespread sharing of longevity data, researchers at the European Bioinformatics Institute developed the SurvCurv database, which provides free and open survival data of model organisms from published studies as well as basic online analysis tools. SurvCurv data on *Drosophila* aging were used to characterize the variability of longevity experiments and identified significant life span effects from experimental tools of the UAS/GAL4-expression system usually thought to be inert (*Aging Cell*, 12:917-22, 2013). Even larger quantities of longevity data in model organisms are on the horizon as high-throughput methods for automated determination of life spans in *C. elegans* are developed.

> Usually only processed results of the analysis or a graphical representation thereof are published, while the original data are not made available.

In addition to collating and sharing these data, complete annotation of the experimental conditions and procedures is important for the field to be able to make the most of the growing number of aging experiments. In other areas of life science, minimal information guidelines, which specify essential annotation that should be reported with each data set, have proven to be a successful approach to raise awareness and improve the quality of annotations. For survival data, work on a minimal information reporting guideline began last year, when I drafted an initial proposal and conducted a survey among researchers working on aging about their experiences with survival-data reporting and their opinion on the importance of annotation categories. The results are currently being evaluated and discussed by an international group of researchers, and a revised proposal for a minimal information reporting guideline is being prepared. As with all community efforts, the more contributors and supporters there are, the higher the benefit for everyone. So let's share and make the most of longevity data to advance our understanding of aging. ■

Matthias Ziehm is a postdoctoral researcher at University College London and at the EMBL European Bioinformatics Institute outside Cambridge.

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Quantity or Quality?

Living longer doesn't necessarily mean living healthier.

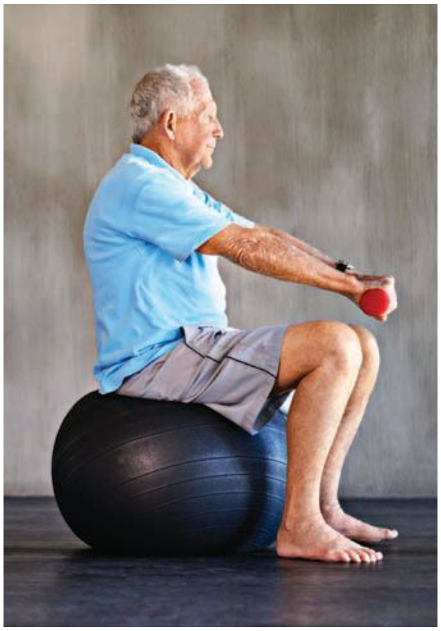
BY ANKITA BANSAL AND HEIDI A. TISSENBAUM

Aging can be simply defined as changes over time in the biological functioning of an organism. These age-related changes are often responsible for the decline of immune, muscular, cognitive, and metabolic function, which ultimately leads to death of the organism. In humans, aging is also accompanied by the higher risk of diseases such as Alzheimer's, cancer, and heart disease. These physiological signs of aging are difficult to measure, and current research on aging typically relies on mortality rates or measuring life span to assess the impacts of treatments, regimens, or genetic mutation on aging. However, these metrics do not adequately equate to the aging process and give little information regarding the health of the individuals or the population.

In the nematode *Caenorhabditis elegans*, for example, a model organism commonly used in aging research, alteration of single genes involved in any of four pathways (insulin signaling, dietary restriction, inhibition of translation, and the electron-transport-chain complex) can lead to significant life span extension. But do animals with such mutations stay healthy as they age, or is there a physiological cost to increased longevity? It is often assumed that an increase in life span will also lead to increased health span, but this has not been evaluated comprehensively.

In a recent study, we tried to answer this question by comparing changes in the health of the long-lived *C. elegans* with that of wild-type worms, examining multiple aspects of health over the life of the worms. Specifically, we examined established reproducible parameters that declined with age, including the ability to move and to maintain homeostasis. We assessed the ability to move using two methods. First, we placed individual animals on solid media and let them move for five minutes, then used image-analysis software to measure the distance traveled. In the second analysis, we put animals in liquid media and filmed their movement for 30 seconds, noting both the number of body bends and the coordination of the movement. To test the animals' ability to maintain homeostasis, we measured response to two different external stressors: heat and oxidative stress. We then calculated the number of days the animals spent in a healthy state (health span) or in a state of frailty (gerospan). We also modeled the data to determine the rate of decline in each health assay.

Our results revealed that some of the long-lived worms stayed healthy for a longer period than wild-type animals when measured chronologically (by simply counting the number of days). However, all long-lived mutants also spent an increased number of days in a frail state, showing little movement and high sensitivity to external stress. This suggests that a consequence of increasing the number of healthy days is a prolonged number of days spent in a frail state. Moreover, when we calculated the frail period as a proportion of animals' maximum life span for a given strain, the data looked much worse: there was little health extension but a prolonged period of frailty (*PNAS*, doi:10.1073/pnas.1412192112, 2015).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Mortality rates and life span do not adequately equate to the aging process and give little information regarding the health of the individuals or the population.

These data reveal a significant physiological cost to life span extension in *C. elegans* and that life span and health span can, in fact, be uncoupled. Although extensive health span studies have not been done in other organisms, long-lived dietary-restricted and insulin growth factor-1–deficient mice do exhibit extended health costs such as delayed sexual maturity, reduced fertility, and reduced muscle strength (*Science*, 299:1342-46, 2003; *Mech Ageing Dev*, 126:938-50, 2005). Taken together, these data point out the necessity of examining multiple health span parameters and not simply life span for future aging research. ■

Ankita Bansal, now a postdoc at the University of Pennsylvania, completed her PhD in April 2014 in the University of Massachusetts Medical School lab of Heidi Tissenbaum, a William Randolph Hearst Young Investigator and a professor in the Department of Molecular, Cellular and Cancer Biology and the Program in Molecular Medicine.

Tricky Transfections

A combination of microinjection and electroporation inserts genes into hard-to-reach cells.

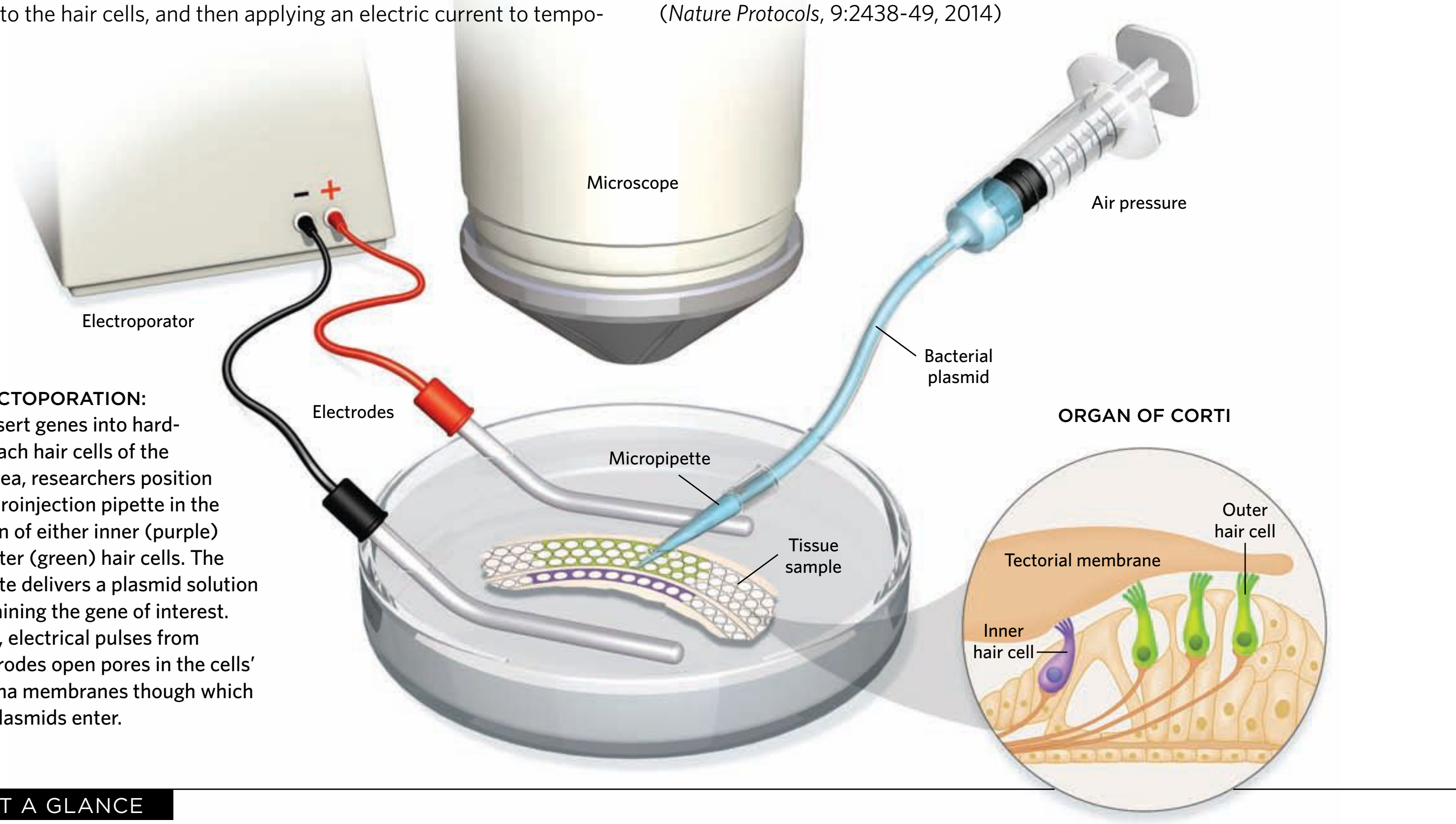
BY RUTH WILLIAMS

The study of human hearing—how it develops, functions, and can diminish with age or disease—would be a great deal easier if only the hair cells of the ear weren't so difficult to get at. These mechanosenory cells, which convert sound-wave vibrations into electrical signals for the auditory nerves, are buried deep within the organ of Corti in the cochlea and are largely inaccessible when it comes to transfecting genes for functional studies, even when the tissue is cultured.

Indeed, with the exception of laborious viral vector–based delivery, standard transfection approaches "just don't work," says hearing researcher Tony Ricci of Stanford University. But now, Ulrich Müller and his team from the Scripps Research Institute have found that by dissecting out the cochlear duct, injecting the genetic payload right next to the hair cells, and then applying an electric current to temporarily create pores in the hair cells' plasma membranes (electroporation), they can succeed where other transfection methods have failed.

"It's very exciting because it fills a gap, technically, in what we are able to do when trying to investigate and manipulate these auditory sensory cells," says Ricci. For gene-function studies, he says, you often "had to make a mouse," which can take up to a year. But now, "you can basically use 'injectoporation' as a tool for screening molecular interactions and then you can decide if you want to make a mouse later," he says.

Müller has already used the technique to decipher components of the elusive hair-cell mechanotransduction machinery, but says that, in principle, injectoporation could be used for any tissue of the body "where access of the plasmid to the cells of interest is limited." (*Nature Protocols*, 9:2438-49, 2014)



INJECTOPORATION: To insert genes into hard-to-reach hair cells of the cochlea, researchers position a microinjection pipette in the region of either inner (purple) or outer (green) hair cells. The pipette delivers a plasmid solution containing the gene of interest. Then, electrical pulses from electrodes open pores in the cells' plasma membranes though which the plasmids enter.

AT A GLANCE

TECHNIQUE	HOW IT WORKS	PRODUCTION TIME	MAXIMUM INSERT SIZE	USE IN VIVO
Viral transfection	Gene of interest is inserted into a viral vector, which is used to infect hair cells.	Several weeks	Typically 5 Kb	Yes
Injectoporation	DNA, RNA, or even proteins, in principle, are injected into the vicinity of hair cells in cultured cochlear-duct cells using a micropipette. Sample is then electroporated.	Practically none. DNA can be loaded directly into pipette.	Longest so far: 10 Kb	No

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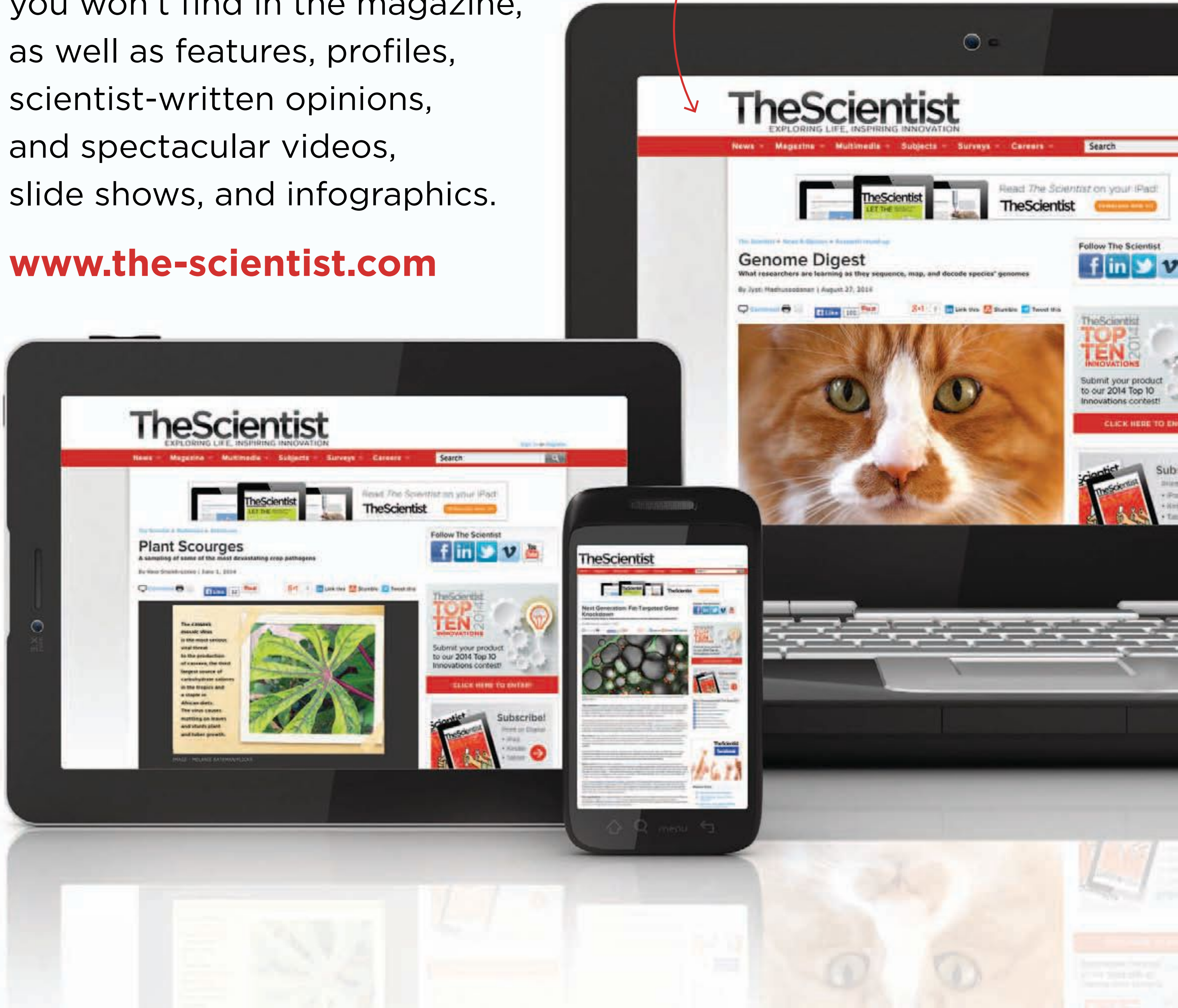
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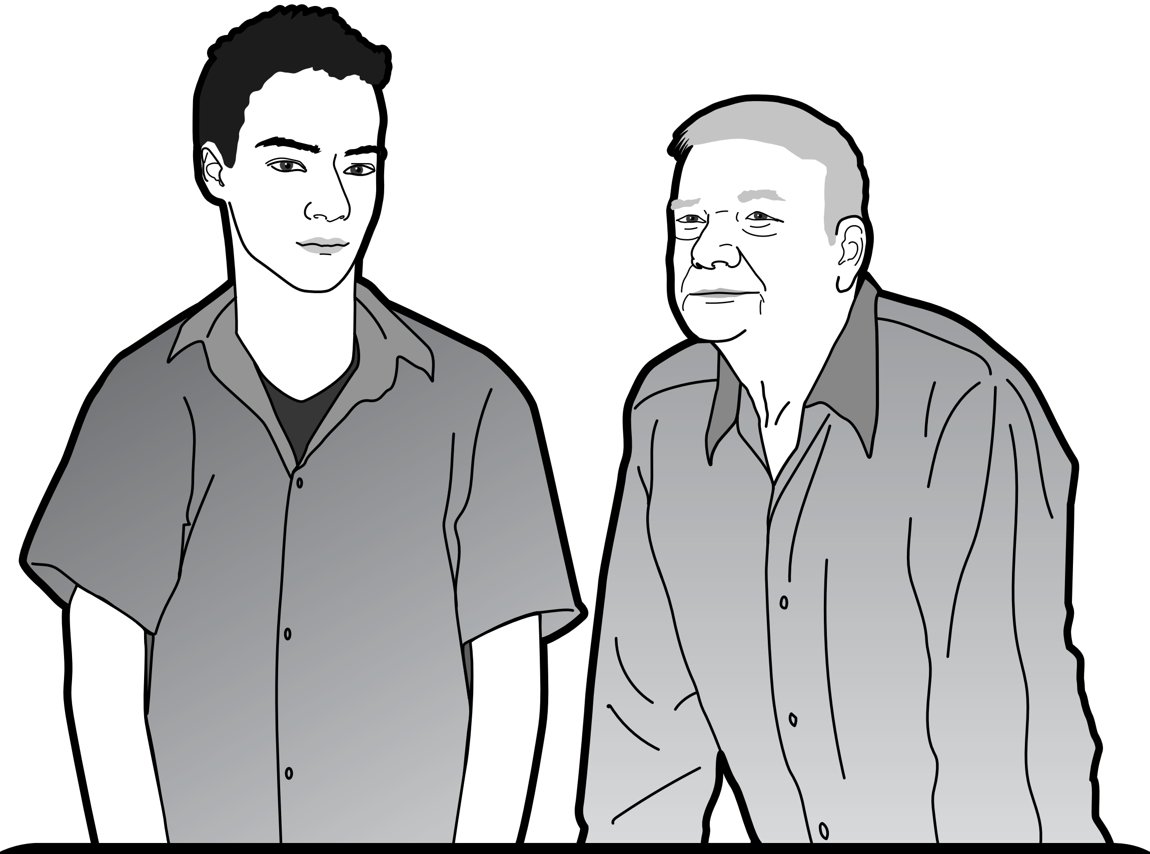

HOW WE AGE

From DNA damage to cellular miscommunication, aging is a mysterious and multifarious process.

BY *THE SCIENTIST* STAFF

Growing old is a fact of life. And there's no mistaking it, given the increased fatigue, weakened bones, and ill health that generally accompany aging. Indeed, age is the number one risk factor for myriad diseases, including Alzheimer's, cancer, cataracts, and macular degeneration. And while researchers are making progress in understanding and treating each of these ailments, huge gaps remain in our understanding of the aging process itself.

"We age so completely and in so many different ways," says stem cell biologist Derrick Rossi of Harvard University. "We are programmed to die."

The aging process can be traced down to the level of cells, which themselves die or enter senescence as they age, and even to the genomic level. Accumulation of mutations and impairments in DNA repair processes are highly associated with symptoms of aging. In fact, disorders that cause premature aging are typically caused by mutations in genes involved in the maintenance of our DNA. And at the cellular level, decreases in stem cells' proliferative abilities, impairments in mitochondrial function, and proneness to protein misfolding can all contribute to aging. As scientists continue to detail these various processes, says Paul Robbins of the Scripps Research Institute, "the big question is, 'At what step along all these pathways is the best place to intervene to try to promote healthy aging?'"

While diverse strategies—from caloric restriction to genetic manipulation—have proven to extend life span in model organisms in the lab, these animals are not necessarily enjoying longer periods of health. (See "Quantity or Quality?" on page 30.) In the end, researchers studying aging must learn not just how to extend life, but how to prevent age-related disease and physical decline.

"The [therapeutic] goal would be to increase health span, not life span," says Rossi. "There's nothing fun about living to be really old if your health diminishes to the point that it's no longer fun to be alive."

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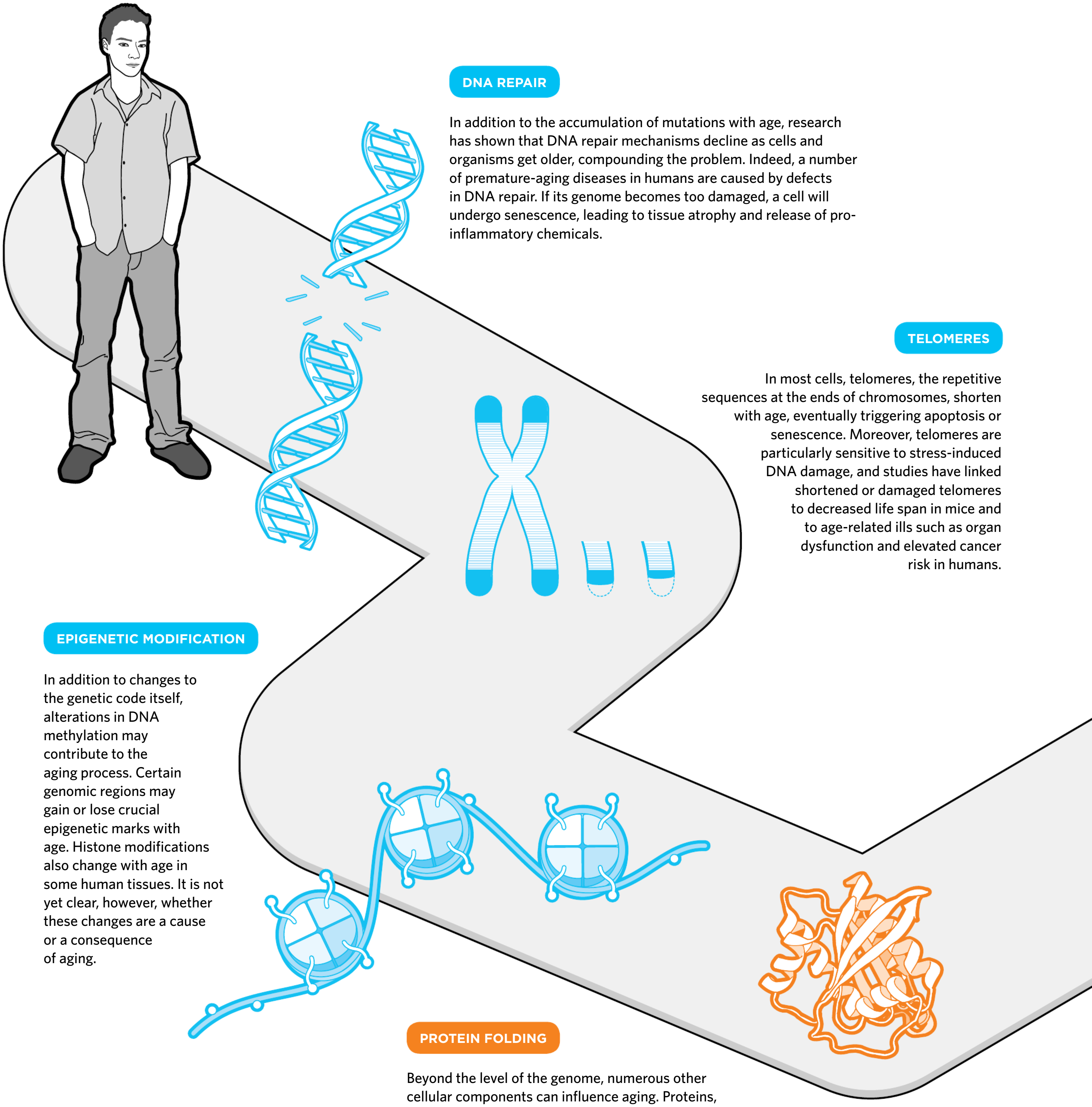

DNA REPAIR

In addition to the accumulation of mutations with age, research has shown that DNA repair mechanisms decline as cells and organisms get older, compounding the problem. Indeed, a number of premature-aging diseases in humans are caused by defects in DNA repair. If its genome becomes too damaged, a cell will undergo senescence, leading to tissue atrophy and release of pro-inflammatory chemicals.

TELOMERES

In most cells, telomeres, the repetitive sequences at the ends of chromosomes, shorten with age, eventually triggering apoptosis or senescence. Moreover, telomeres are particularly sensitive to stress-induced DNA damage, and studies have linked shortened or damaged telomeres to decreased life span in mice and to age-related ills such as organ dysfunction and elevated cancer risk in humans.

EPIGENETIC MODIFICATION

In addition to changes to the genetic code itself, alterations in DNA methylation may contribute to the aging process. Certain genomic regions may gain or lose crucial epigenetic marks with age. Histone modifications also change with age in some human tissues. It is not yet clear, however, whether these changes are a cause or a consequence of aging.

PROTEIN FOLDING

Beyond the level of the genome, numerous other cellular components can influence aging. Proteins, for example, whose function is dependent on a specific conformation, are more likely to be misfolded in older cells than younger ones, though it is still unclear whether these changes lead to aging processes or are merely a consequence of them. Aging also seems related to declines in the production of molecular chaperones that help fold proteins and in the functioning of pathways that clear misfolded proteins.

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AS TIME GOES BY

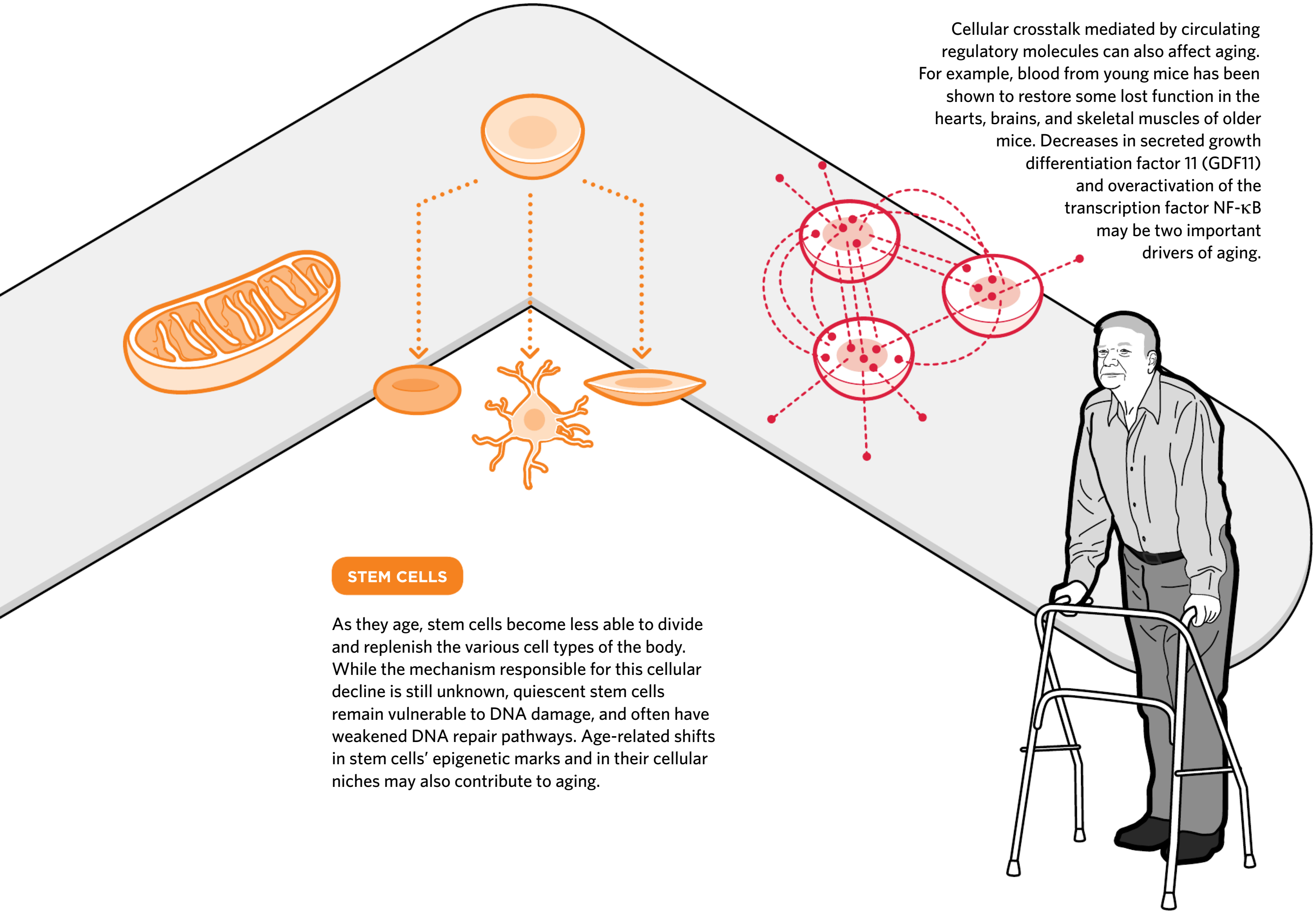
Aging is the outcome of diverse and complex changes in normal biological functions, from the accumulation of DNA damage to dysfunction of proteins and altered communication both within cells and among distant tissues in the body. Researchers are beginning to piece together how we age at the level of our genomes, our cells, and our whole bodies, in hopes of identifying strategies for slowing decline and extending healthy life span.

MITOCHONDRIAL FUNCTION

Reactive oxygen species (ROS) wreak havoc on a cell and have been proposed as one of the many drivers of aging. As primary producers of ROS, mitochondria have long been presumed to contribute to age-related damage. But growing evidence that some level of ROS signaling is critical for normal physiology has researchers restructuring their view of the organelle's role in aging.

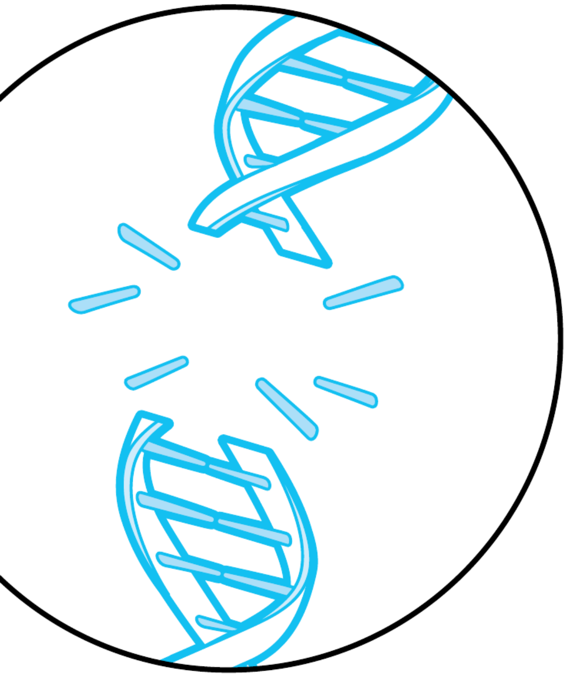
CELLULAR COMMUNICATION

Cellular crosstalk mediated by circulating regulatory molecules can also affect aging. For example, blood from young mice has been shown to restore some lost function in the hearts, brains, and skeletal muscles of older mice. Decreases in secreted growth differentiation factor 11 (GDF11) and overactivation of the transcription factor NF-κB may be two important drivers of aging.



STEM CELLS

As they age, stem cells become less able to divide and replenish the various cell types of the body. While the mechanism responsible for this cellular decline is still unknown, quiescent stem cells remain vulnerable to DNA damage, and often have weakened DNA repair pathways. Age-related shifts in stem cells' epigenetic marks and in their cellular niches may also contribute to aging.



DAMAGE CONTROL

As DNA replicates, the cellular machinery involved in the process makes mistakes, leading to changes in the DNA sequence. Mutagens such as reactive oxygen species (ROS) or UV radiation can also damage DNA. Most of the time, DNA repair mechanisms fix the damage, but errors slip through and accumulate as an organism ages. Aging has also been linked to the deterioration of DNA repair machinery, allowing permanent errors to become more common in older organisms.

Once DNA has become too damaged, cells kill themselves or enter a nonreplicating state, a process called senescence. Loss of cells can lead to tissue atrophy and dysfunction. And senescent cells, though largely dormant, may actually speed the aging process by secreting inflammatory cytokines thought to contribute to atherosclerosis and other aging-related diseases. Additionally, DNA scaffolding proteins that typically help stabilize the genome show changes with age, contributing to impaired cell division, increased senescence, and other aging-related processes.

While it's unclear exactly how DNA damage contributes to aging, what's certain is that the damage and mutations contribute to cancer, says Jan Vijg, a geneticist at Albert Einstein College of Medicine in New York City. "There is this exponential increase in cancer risk during aging, so it's not at all unlikely . . . that accumulation of damage to the genome is really a major factor here," he says.

Premature-aging diseases in humans also point to the role of DNA repair and stabilization mechanisms in the aging process. For instance, people with Hutchinson-Gilford progeria syndrome have mutations in a gene encoding scaffolding proteins called nuclear lamins and suffer from hair loss, an aged appearance, vision deterioration, and atherosclerosis as children. In another example, Werner syndrome patients, who develop symptoms of advanced aging as teenagers, have mutations in a gene involved in DNA repair. (See "Nourishing the Aging Brain" page 46.)

But how DNA damage leads to aging in normal adults remains an open question. "We have beautiful next-generation sequencing methods, and we can sequence the DNA that we isolate from a tissue. But that will not help us a lot because mutations are random, and they will be different from cell to cell," says Vijg, who is now trying to understand how such mosaics of cells work together to cause aging.

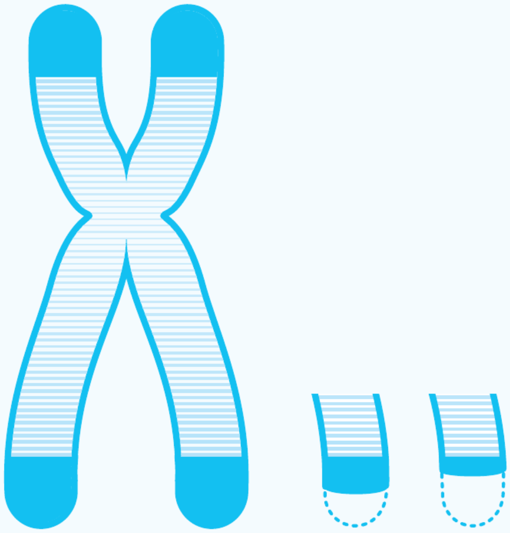
—Kate Yandell

Aging has been linked to the deterioration of DNA repair machinery, allowing permanent errors to become more common in older organisms.

TELOMERE TROUBLE

A particularly influential form of DNA damage occurs at telomeres, the repetitive sequences that cap chromosomes and shorten with age. While germ and stem cells express an enzyme called telomerase that replenishes telomeres, most cells' telomeres shrink with every division, due to the fact that DNA polymerase cannot fully replicate the ends of chromosomes. If the telomeres shrink too much or are damaged, cells undergo apoptosis or enter senescence.

Telomere damage has clear effects on aging. Mice with short telomeres have diminished life spans and reduced stem-cell and organ function, while mice whose telomerase is enhanced in



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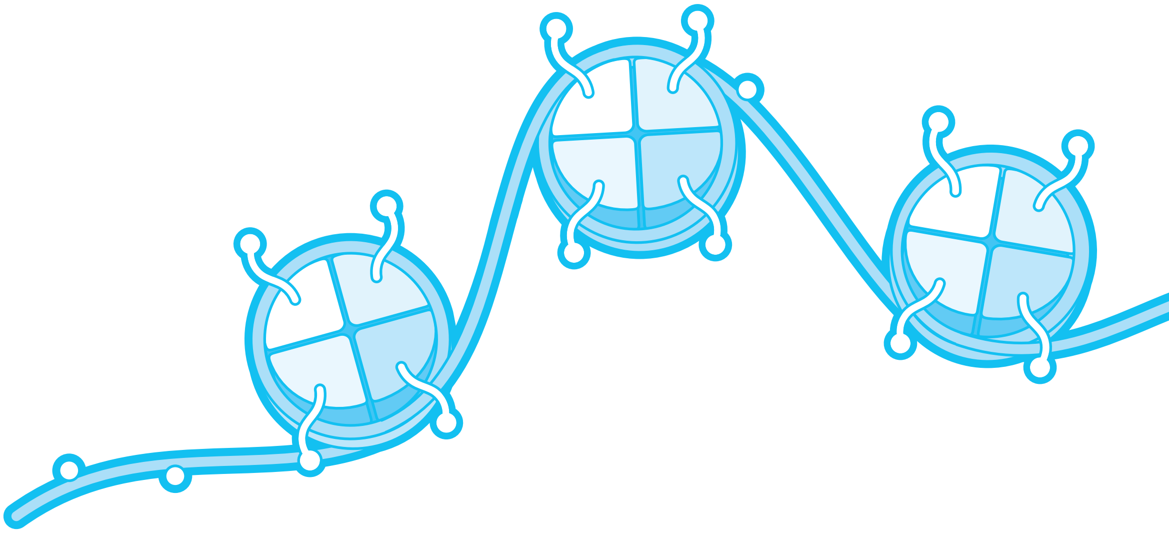

EPIGENETIC SHIFTS

In the early 1990s, Jean-Pierre Issa, then at Johns Hopkins University, was studying changes in DNA methylation in colon cancer cells when he noticed that the epigenetic marks were shifting over time—not just in tumor cells, but also, to a lesser degree, in a variety of healthy cells. Indeed, mapping of DNA methylation in human cells has shown that some areas of the genome become hypermethylated with age, while others show reduced methylation. Histone modifications, another type of epigenetic mark, have also been shown to change with age in some human tissues.

These changes happen through mistakes during the processes of replication or DNA damage repair. During replication, DNA methylation and histone modifications are not always perfectly reproduced. When DNA is damaged, repair proteins must often remove epigenetic marks to access the damaged genetic material and repair it. Epigenetic marks can then be omitted or replaced incorrectly.

The question now is whether these epigenetic changes influence aging. "Is this an epiphenomenon that happens just because we age, or is it actually causing symptoms or diseases of aging and limiting life span?" says Issa, who now studies the epigenetics of cancer and aging at Temple University in Philadelphia, Pennsylvania.

Epigenetic changes are known to contribute to cancer, and there is intriguing evidence from animal models that changes to histone modifications do affect aging. For instance, inhibiting a histone demethylase enzyme extends life span in *Caenorhabditis elegans* (*Cell Metab*, 14:161-72, 2011), while alterations to proteins involved in methylating histones leads to longer-lived flies (*PNAS*, 107:169-74, 2010) and worms (*Nature*, 466:383-87, 2010). Similarly, altering acetylation can affect life span in yeast. (See "Weiwei Dang: Epigenetics in Aging," page 57.) Issa is currently searching for drugs that can modulate DNA methylation in cancer and hopes they may one day slow aging.

But DNA methylation changes with age are not uniform, he notes. "We gain at some sites, and we lose at other sites," Issa says. Simply deleting or overexpressing methyltransferases will be insufficient to recapitulate the methylation patterns of youth.

—Kate Yandell

adulthood age more slowly (*EMBO Mol Med*, 4:691-704, 2012). In humans, mutated telomerase is associated with disorders involving organ dysfunction and elevated cancer risk (*J Clin Invest*, 123:996-1002, 2013).

In recent years, researchers have also shown that telomeres are targets of stress-induced DNA damage (*Nat Comm*, 3:708, 2012). "For reasons we don't really understand yet, they are very sensitive to external stress, more than the rest of the genome," says João Passos, a researcher at Newcastle University's Institute for Ageing in the U.K.

Once telomeres have been damaged, they are difficult to repair. They protect chromosomes from fusing with one another by recruiting protein complexes called shelterins that prevent overzealous DNA repair proteins from mistaking loose ends for double-strand breaks. This may also prevent repair proteins from accessing legitimate DNA damage, however, leading to cell death or senescence.

Telomeres may be especially prone to DNA damage in order to protect the body from cancer, Passos suggests. Because they are disproportionately damaged by stressors, and because telomere damage so often leads to senescence, they could be like canaries in coal mines, warning cells that carcinogens are present. Telomeres may, in fact, be DNA-damage sensors that shut down cell proliferation in times of stress, Passos says. This is a double-edged sword, as senescence lowers cancer risk but also leads to symptoms of aging.

—Kate Yandell

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IN THE FOLDS

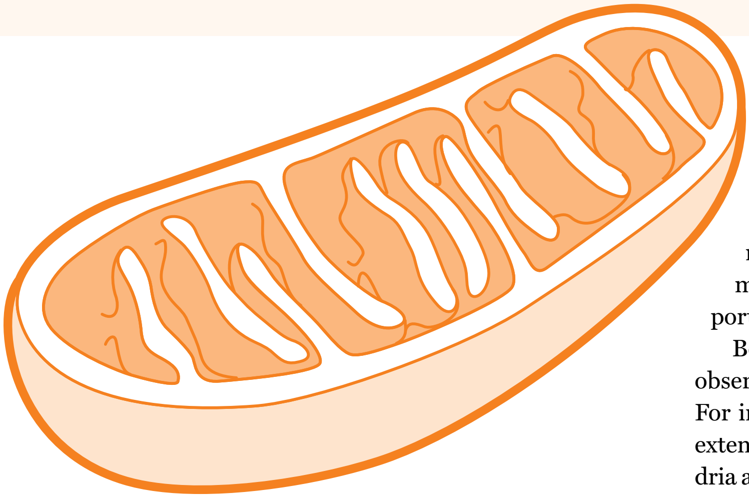
Life depends on proper protein function. And proper protein function is all about proper protein folding. Misshapen proteins are often rendered useless and can clump together with other misfolded proteins inside cells. It is not yet clear whether protein misfolding leads to aging, but it appears that it is an almost inevitable physiological reality that the two coincide. To add insult to injury, advancing age also brings about the decline of molecular chaperones that aid in the folding process and of protective pathways that normally help clear misfolded proteins from cells.

"The big open question is whether the accumulation of misfolded protein aggregates is the cause or consequence of the aging process," says Claudio Soto, a neuroscientist at the University of Texas Health Science Center at Houston who studies the effects of misfolded protein aggregations in the brain. "The hypothesis is that maybe there is a widespread accumulation of misfolded protein aggregates affecting all cells in the body, and that produces progressive dysfunction of cells in the body that leads to aging."

The model organism *C. elegans* has yielded tantalizing clues that may help answer the chicken-or-egg question regarding protein misfolding and aging. Northwestern University molecular biologist Richard Morimoto and colleagues showed that the worm's proteostasis machinery, which includes molecular chaperones, stress-response transcription factors, and protein-degrading enzymes, starts breaking down very early on in the animal's three-week life span (*PNAS*, 106:14914-19, 2009). "What's interesting is that this happens very early in adulthood," Morimoto says. "You see these changes within days of becoming an adult."

Soto says that problems with protein folding might be central to the multitude of molecular deficiencies that characterize an aging body. After all, normal protein folding is necessary for gene expression, enzyme function, and a host of other crucial physiological events. "This could actually unify the different processes," he says.

And if protein misfolding does act as a sort of linchpin in aging, correcting it may be a way of staving off a host of age-related maladies or even aging itself, Soto adds.



THE GOLDILOCKS ORGANELLE

The free-radical theory of aging, developed in the 1950s, proposes that reactive oxygen species (ROS) cause aging by wreaking global cellular damage. As one of the major sources of ROS, mitochondria—and, specifically, ROS injuries to these organelles and their DNA—are presumed to also play a role in aging. "It's one of the robust theories of aging," says Gerald Shadel, who studies mitochondria at Yale University. Often, he says, it's what comes to mind first when people think of the molecular and cellular mechanisms of aging. And while there is some evidence supporting it, "there's now a lot of evidence against that concept."

Beginning in the 1990s, scientists studying model organisms observed phenomena that contradicted the free radical theory. For instance, enzymes that block the production of ROS didn't extend the life span of mice; in worms, stressing the mitochondria at a certain stage of development actually increased life span; and, as Shadel's group showed in 2011, ramping up mitochondrial ROS extended longevity in yeast (*Cell Metab*, 13:668-78, 2011). "It looks like ROS signaling is important for normal physiology," says Shadel.

Such evidence is helping to shape a new view of oxidative damage to mitochondria. "If damage is not too severe, there's some sort of protective response," says Toren Finkel, an aging researcher at the National Heart, Lung, and Blood Institute. "What won't kill you makes you stronger."

There is a limit to how much damage the organelle can handle, however, and mitochondrial dysfunction may well contrib-

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"The good news is that, if that's the case, you could envision really intervening in this and delaying the aging process."

—Bob Grant

Beginning in the 1990s, scientists studying model organisms observed phenomena that contradicted the free radical theory.

ute to aging. Recent evidence in mice shows that mutations in mitochondrial DNA are linked with shortened life span (*Sci Rep*, 4:6569, 2014). "It's consistent with this idea that maybe from metabolism you get oxidative stress, you then get DNA damage, then that decline in mitochondrial function makes us age," says Finkel. "I think there's still a lot to that [idea]."

Shadel says mitochondria's role in aging is likely not limited to ROS or even DNA damage. Given the organelles' broad-reaching involvement in metabolism, inflammation, and epigenetic regulation of nuclear DNA, Shadel says, "I think they're central integrators of many of the pathways we've implicated in aging."

—Kerry Grens

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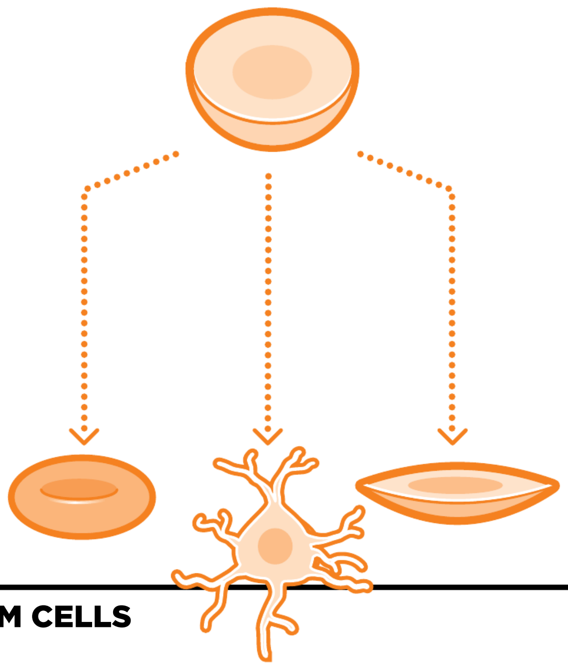


STEM CELLS

Healthy adults produce about 200 billion new red blood cells each day to replace the same number removed from circulation every 24 hours. But the rate of blood-cell production declines with age. For this and other reasons, around 10 percent of people age 65 and older are anemic. Scientists are now homing in on how hematopoietic stem cells (HSCs) and other stem-cell populations show reduced regenerative capacity with age. (See "In Old Blood," *The Scientist*, August 2014.)

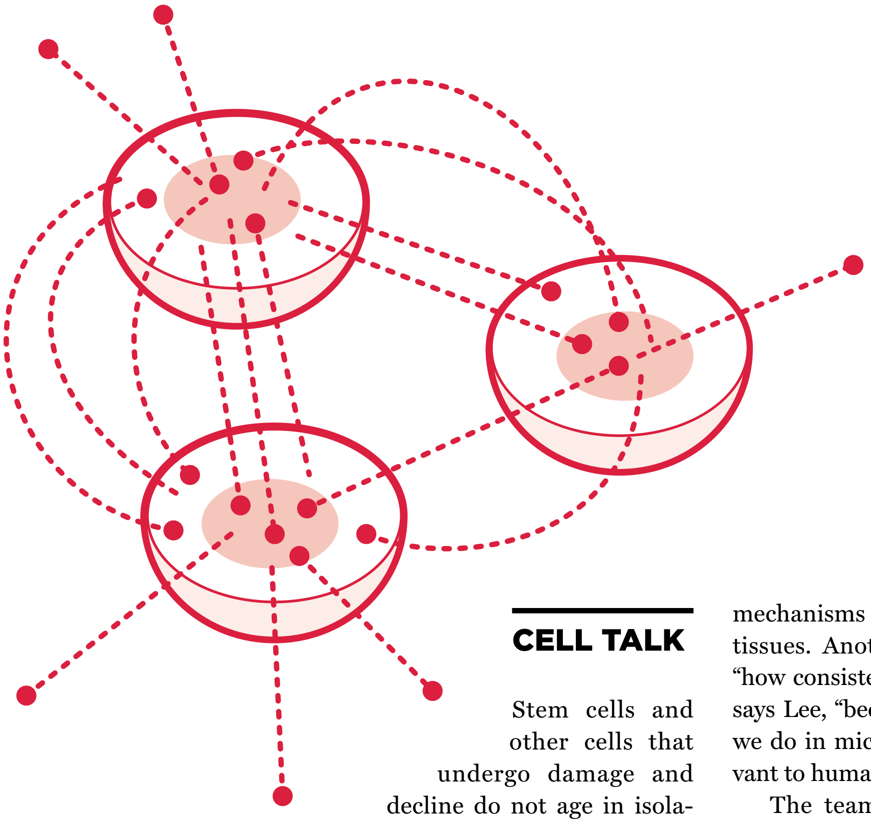
"It's a bit of a mystery as to why these self-renewing cells in different tissues stop working," says clinical geneticist Norman Sharpless from the University of North Carolina at Chapel Hill School of Medicine. "The nature of molecular aging at the cellular level is not fully known."

While HSCs remain dormant, or quiescent, for extended periods of time, they remain vulnerable to DNA damage. And during these periods of dormancy in mouse HSCs, DNA damage response and repair pathways weaken, Harvard's Derrick Rossi and his colleagues reported recently (*Cell Stem Cell*, 15:37-50, 2014). This reduced capacity for DNA damage repair can let harmful mutations linger. "What we found is that this [cellular] life of luxury on the couch is about as detrimental to the health of an HSC as life perpetually spent on the couch [is to] humans," Rossi says.

Researchers have also linked epigenetic alterations, such as locus-specific changes in DNA methylation, to the reduced regenerative capacity of stem cells with age. And age-related shifts in the environment in which stem cells divide and differentiate, dubbed the stem-cell niche, may also contribute to stem-cell aging. For example, as Hartmut Geiger of the University of Ulm, Germany, and his colleagues showed in 2012, age-related changes in supportive niche cells influence hematopoietic progenitor cell populations: young microenvironments fostered more homogeneous groups of cells as compared with aged ones (*PLOS ONE*, doi:10.1371/journal.pone.0042080, 2012).

Exactly why and how stem cells slow down with age is still a mystery. "Everybody's got a favorite theory," says Sharpless, but "it's sort of an open question."

—Tracy Vence



CELL TALK

Stem cells and other cells that undergo damage and decline do not age in isolation. Researchers are finding that some processes of aging influence the release of regulators that circulate in the blood. “At one time, everybody thought, well, cells just get old and die,” says Paul Robbins of the Scripps Research Institute. “But the cells do more than just die. They do negative things, and they persist.”

One such regulator is growth differentiation factor 11 (GDF11), which controls the gene expression patterns that set up front-to-back orientation in mammalian embryos and measurably decreases with age. Recently, a team of Harvard Medical School researchers surgically joined young and old mice—a classical technique called parabiosis—to investigate the roles of blood-borne factors in aging. Amy Wagers, Richard Lee, and their colleagues found that young blood can restore some lost functions in the hearts, brains, and skeletal muscles of older mice, and that these effects can be replicated by treating old mice with GDF11 (*Cell*, 153:828-39, 2013; *Science*, 344:630-34; 344:649-52, 2014).

The researchers are now working to pinpoint the sources of circulating GDF11, as well as to understand the mechanisms by which it remodels aging tissues. Another important question is “how consistent this is across mammals,” says Lee, “because then these things that we do in mice might become more relevant to humans.”

The team is collecting blood samples from mammals of different ages—“everything from cats to cows” and other farm animals, says Lee—to examine their GDF11 levels. They also hope to develop a more sensitive method to measure the protein in humans, in order to test associations between GDF11 levels and aging-related diseases.

Other scientists are focusing on the transcription factor NF-κB, a central activator of inflammation, as a driver of aging. Overactivation of NF-κB may cause senescent cells to release cytokines that stimulate inflammation and lead to further degeneration, even in far-flung parts of the body. “It seems that with almost anything that activates NF-κB, if you reduce it, it improves aging,” says Robbins. He and his colleagues have demonstrated that inhibiting NF-κB can stave off cell senescence in mice that age prematurely due to DNA repair defects (*J Clin Invest*, 122:2601-12, 2012). “[We want] to see if we can understand the contribution of what goes on within a cell versus the contribution of what that cell secretes that affects cells at a distance,” he says.

—Molly Sharlach

> We want to see if we can understand the contribution of what goes on within a cell versus the contribution of what that cell secretes that affects cells at a distance.
>
> —Paul Robbins,
> Scripps Research Institute

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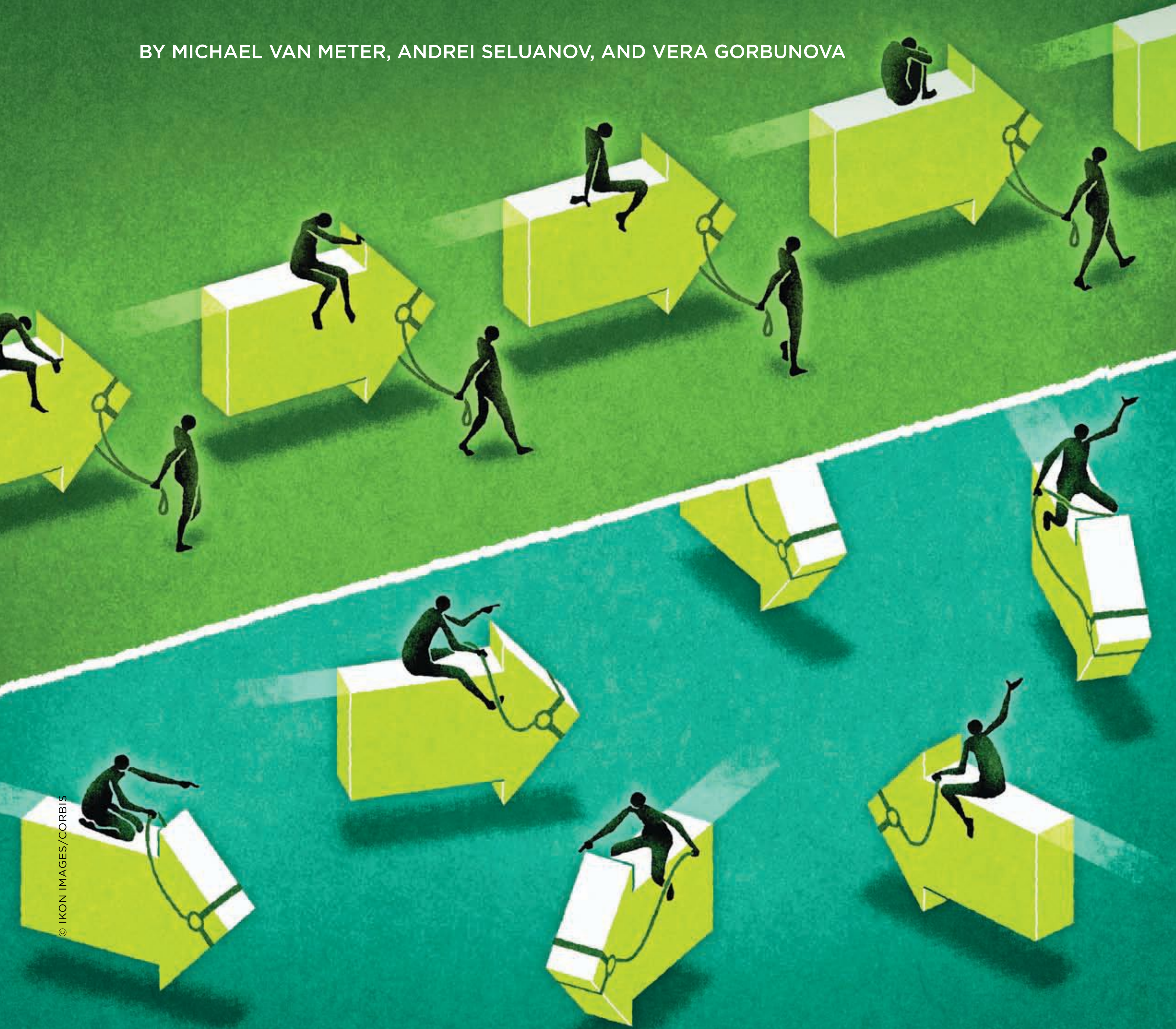
Wrangling Retrotransposons

These mobile genetic elements can wreak havoc on the genome. Researchers are now trying to understand how such activity contributes to the aging process.

BY MICHAEL VAN METER, ANDREI SELUANOV, AND VERA GORBUNOVA



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Genomes are hotbeds of evolutionary conflict. Perhaps nothing speaks to this idea better than the war raging between retrotransposons and their host genomes. Retrotransposons, often referred to as jumping genes, are mobile genetic elements that parasitize host machinery to replicate themselves across the genome. Since their emergence more than 100 million years ago, retrotransposons have been enormously successful. Modern mammalian genomes, for example, are riddled with the scars of these copy-and-paste events, with retrotransposon-derived DNA now accounting for nearly 50 percent of the human genome.

The most dangerous retrotransposon in mammalian genomes is the long interspersed nuclear element-1 (LINE-1 or L1). L1 retrotransposons are a little more than 6 kilobases long and encode an RNA-binding protein and an endonuclease with reverse-transcriptase activity that allow the element to autonomously replicate in the host genome via an RNA intermediate. The human genome contains more than 500,000 copies of L1s. Although the vast majority of these have been inactivated as a result of truncation, mutation, and internal rearrangement, it is estimated that approximately 100 of these L1s per nuclear genome still retain their replication activity. Despite their abundance, however, L1s are not benign. Rather, their activity, and even their presence, represents a real danger to the host, increasing the risk of DNA damage, cancer, and other maladies. Given the consequences of L1 activity, it is unsurprising that host genomes devote considerable resources to suppressing these retrotransposons. Indeed, every step of the L1 life cycle is impeded in some way by host factors such as gene silencing, antiviral defense machinery, small RNAs, and autophagy.

Historically, almost all research on these genomic parasites has focused on their activity in the germline, where any genomic changes will be inherited in the next generation. Little attention has been given to retrotransposition in somatic tissue, because this was thought of as an evolutionary dead end. In recent years, however, evidence has accumulated that L1 elements can become active in a variety of somatic tissues in humans and mice, including in the brain, skeletal muscle, heart, and liver. Intriguingly, some of the highest L1 activity has been observed in aging tissues, particularly those affected by age-related pathologies such as cancer. This raises the interesting possibility that L1 activity may contribute to the aging process.

More and more evidence is accumulating to support this idea. Increased DNA damage and mutagenesis are prevalent in aging tissues, and L1 activity is known to increase following such damage. In addition, a small number of studies have shown that overexpression of L1 can cause cells to senesce, a hallmark of aging tissues. The role of L1 in driving age-related processes is now a topic ripe for study.

Suppressing L1 expression

The most obvious threat posed by L1s is the risk of mutagenic insertions. Depending on where the retrotransposon inserts itself, it can predispose a cell to cancerous transformation, for example. In fact, a hallmark of cancer genomes is increased copy number and heightened activity of L1 retrotransposons. Additionally, multiple human genetic diseases have been reported to stem from L1-driven mutagenesis. But L1s can also wreak havoc on host genomes in a number of other ways. The L1 endonuclease can cause DNA single-strand breaks, for example; the L1 5′ untranslated region (5′ UTR) can deregulate transcription of adjacent host genes; the sheer volume of L1 DNA alone can cause erroneous recombination events. Because genomic instability and DNA damage are key features of aging, it is easy to envision how L1s may contribute to the molecular etiology of the aging process.

Some of the highest L1 activity has been observed in aging tissues, particularly those affected by age-related pathologies such as cancer.

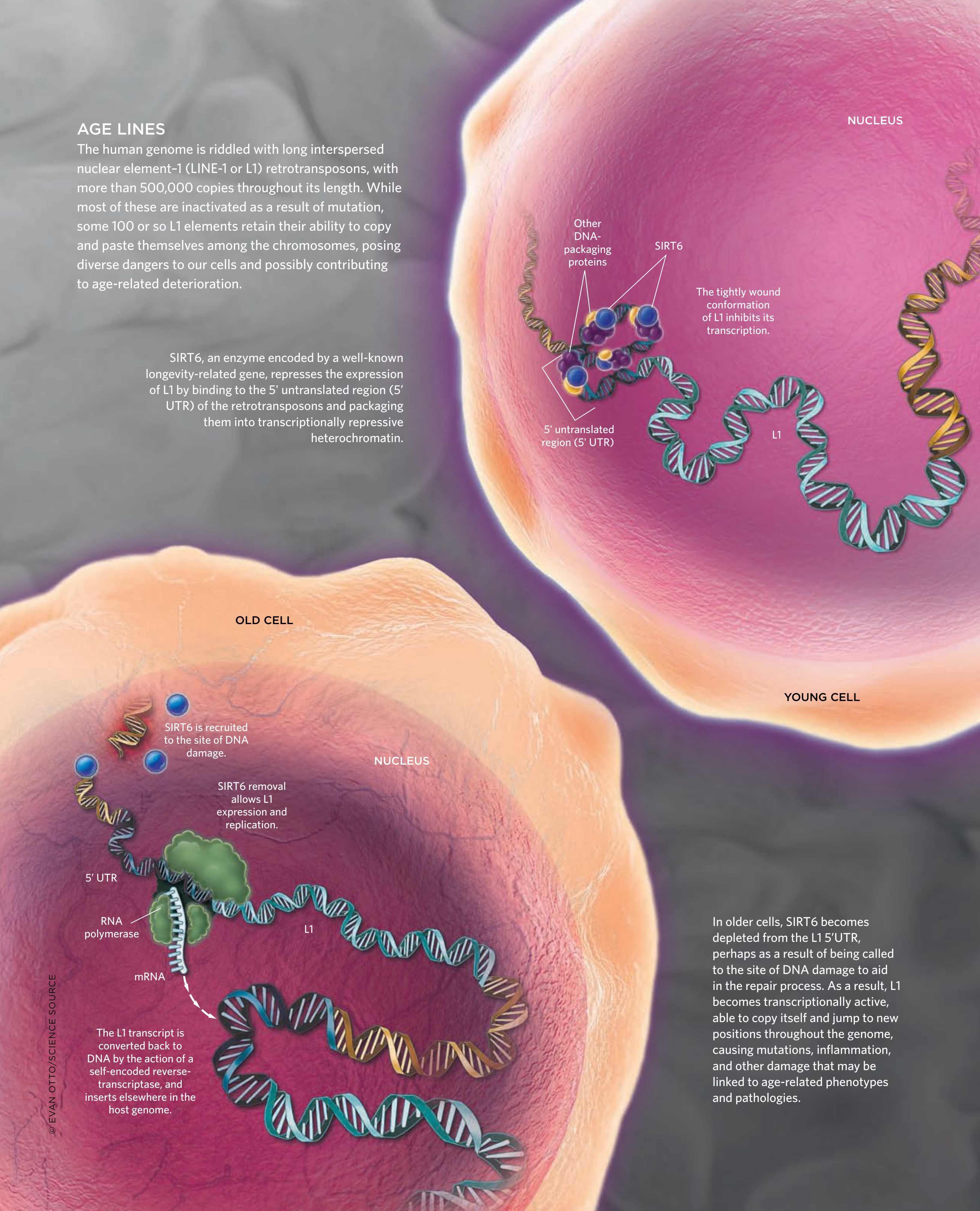
Perhaps the best evidence for the retrotransposon's role in aging comes from the link between the activity of a longevity gene, *SIRT6,* and the repression of L1 in somatic tissues. *SIRT6* encodes an enzyme critical to the forestallment of aging: it maintains telomere length, promotes DNA repair, regulates metabolism, opposes tumorigenesis, and attenuates inflammation—all processes associated with the prevention of age-related decline. Mice lacking *SIRT6* suffer from a severe premature aging syndrome, while mice that overexpress *SIRT6* enjoy extended life spans. Most recently, we found that SIRT6 plays a critical role in keeping L1 silent in young mouse tissues and in presenescent human cells by packaging the retrotransposons into transcriptionally repressive heterochromatin.[1] Specifically, SIRT6 binds to the L1 5′ UTR, where it coordinates the assembly of heterochromatin proteins at the L1 promoter. Once assembled, this silencing machinery saturates the L1 5′ UTR and its associated chromatin with epigenetic modifications that prevent the retrotransposon from being expressed. (See illustration on opposite page.)

Without the ability to express itself, L1 cannot synthesize the parasitic machinery required to initiate its own replication. As a result, the host cell is protected from de novo mutagenesis events. Additionally, because the L1 is heterochromatinized, it has limited ability to interfere with host gene expression or to induce erroneous recombination events. When *SIRT6* is deleted from mouse cells or knocked down in human cells, the L1 5′ UTR escapes heterochomatinization, resulting in massive increases in L1 transcription and de novo L1 insertions.

AGE LINES

The human genome is riddled with long interspersed nuclear element-1 (LINE-1 or L1) retrotransposons, with more than 500,000 copies throughout its length. While most of these are inactivated as a result of mutation, some 100 or so L1 elements retain their ability to copy and paste themselves among the chromosomes, posing diverse dangers to our cells and possibly contributing to age-related deterioration.

SIRT6, an enzyme encoded by a well-known longevity-related gene, represses the expression of L1 by binding to the 5' untranslated region (5' UTR) of the retrotransposons and packaging them into transcriptionally repressive heterochromatin.



The tightly wound conformation of L1 inhibits its transcription.

SIRT6 is recruited to the site of DNA damage.

SIRT6 removal allows L1 expression and replication.

The L1 transcript is converted back to DNA by the action of a self-encoded reverse-transcriptase, and inserts elsewhere in the host genome.

In older cells, SIRT6 becomes depleted from the L1 5'UTR, perhaps as a result of being called to the site of DNA damage to aid in the repair process. As a result, L1 becomes transcriptionally active, able to copy itself and jump to new positions throughout the genome, causing mutations, inflammation, and other damage that may be linked to age-related phenotypes and pathologies.

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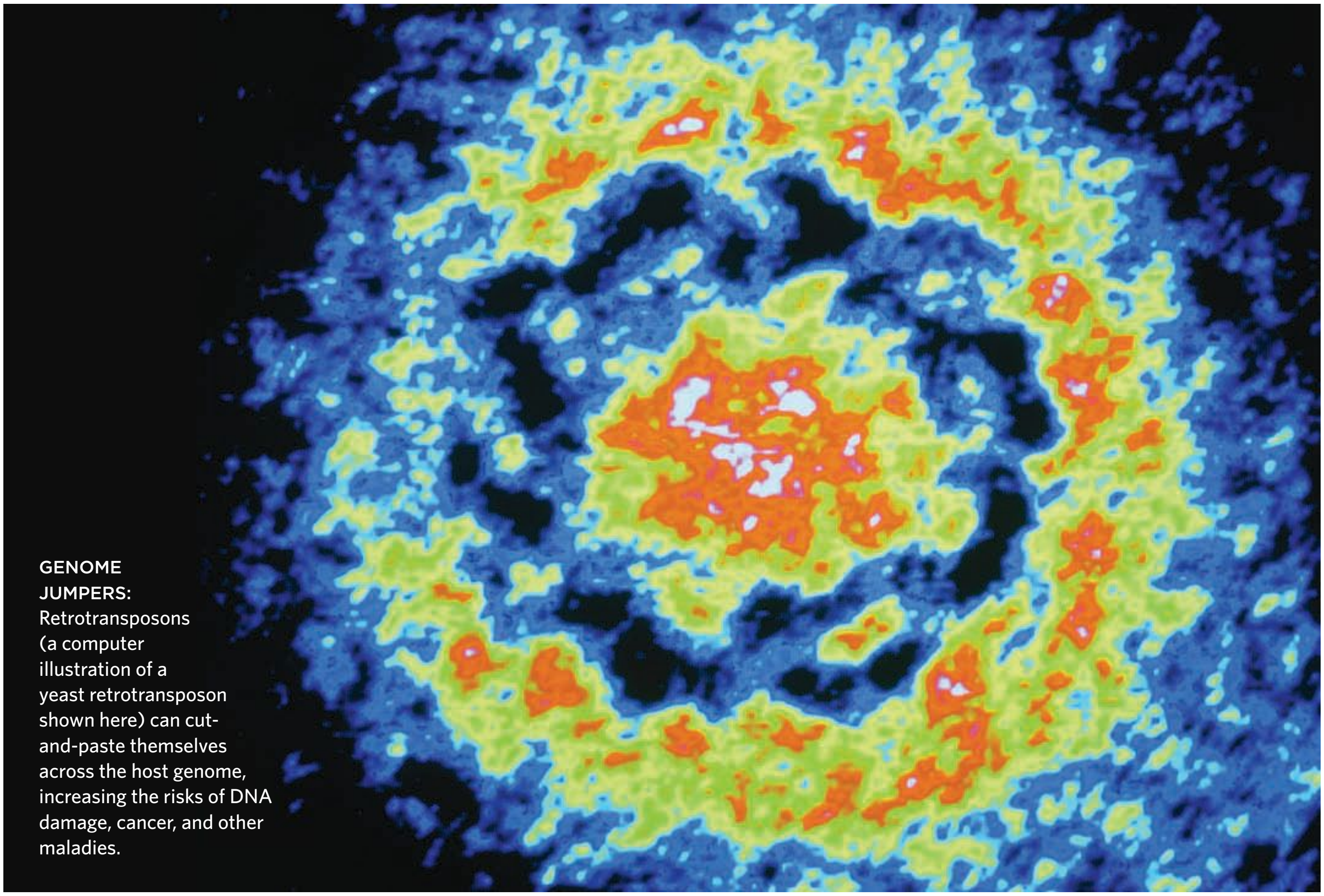
Interestingly, the process by which SIRT6 silences L1 appears to become less efficient with age. During aging, SIRT6 becomes depleted from the L1 5′ UTR, resulting in the de-heterochromatinization of the L1 element. No longer restricted by repressive chromatin, the L1 element becomes transcriptionally active in multiple tissues of the aging mouse, as well as in senescent human cells. But what is happening during aging that prevents SIRT6 from efficiently safeguarding the old cells from these dangerous genomic parasites?

One explanation for this failure may relate to SIRT6's critical role in DNA repair. Several studies have indicated that SIRT6 helps catalyze repair of the damage at numerous types of DNA lesions, including single- and double-strand breaks. A characteristic feature of aging cells is an increase in the amount of DNA damage. It is possible that, under basal conditions, SIRT6 is bound to the L1 promoter, enforcing silencing of the genomic parasite, but upon DNA damage, the enzyme vacates the L1 sequence to facilitate repair at the damage site. This creates a window of opportunity for L1 elements to escape repression and replicate themselves. Consistent with this model, when cells are treated with DNA-damaging agents, such as hydrogen peroxide and paraquat, SIRT6 leaves L1 sites and becomes enriched at damage sites.[1]

Several other factors may also limit the ability of SIRT6 to repress L1s in aging cells. For example, SIRT6 requires a substrate, NAD$^+$, to perform its enzymatic activity. Levels of this metabolite are known to decrease in old cells, likely resulting in decreased efficiency of SIRT6. Moreover, SIRT6 expression levels decrease with age in several cell and tissue types. The reduced availability of the protein may also exacerbate age-related derepression of L1 elements.

Implications for aging

The relocalization of SIRT6 that occurs in a stressed or aging cell is reminiscent of a theory of aging called the relocalization of chromatin modifiers, originally proposed in 1998 by Japanese researchers Hiroaki Kitano and Shinichiro Imai[2] and formalized nearly a decade later by Philipp Oberdoerffer and David Sinclair of Harvard Medical School.[3] This theory postulates that, while mobilization of chromatin-modifying proteins such as SIRT6 in response to stress is beneficial in young organisms, redistribution of these elements may drive deleterious age-related changes. In this regard, the mobilization of SIRT6 to DNA damage sites and subsequent derepression of L1 elements may lead to the manifestation of age-related phenotypes and pathologies. For exam-



GENOME JUMPERS: Retrotransposons (a computer illustration of a yeast retrotransposon shown here) can cut-and-paste themselves across the host genome, increasing the risks of DNA damage, cancer, and other maladies.

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ple, increased L1 activity may contribute to genomic instability—a hallmark phenotype of aging cells—by mutagenizing DNA via de novo insertion events and inducing DNA damage through expression of the L1 endonuclease. Moreover, because DNA damage draws SIRT6 away from L1 sites, this represents the commencement of a malicious cycle: L1-induced DNA damage forces SIRT6 to vacate L1 sites, leading to even higher L1 activity and more DNA damage. Similarly, L1 may be disrupting cellular homeostasis by inducing dysregulation of gene expression and clogging the transcriptional machinery of the host cell. Consistent with these possibilities, overexpression of exogenous L1 is sufficient to drive human fibroblasts and stem cells into stress-induced cellular senescence.[4]

Because genomic instability and DNA damage are key features of aging, it is easy to envision how L1s may contribute to the molecular etiology of the aging process.

The notion that derepression of L1s may be contributing to aging-related pathologies is especially appealing given that brain (in particular, the hippocampus and striatum), liver, and skeletal muscle tissue all appear to be particularly affected by age-dependent increases in L1 activity. Each of these tissues plays a critical role in maintaining organismal homeostasis across multiple signaling pathways, and each exhibits marked declines in function during aging. For example, it is tempting to speculate that increased L1 activity—and the subsequent DNA damage, inflammation, and taxing of the host transcriptional machinery—contributes to the atrophy and insensitivity to insulin signaling that characterize skeletal muscle during aging. Similarly, L1 retrotransposition in the aging brain represents another very intriguing, yet underexplored, mechanism that could explain many of the features associated with the aging nervous system.

Several studies have indicated that de novo insertions of retrotransposons within the nervous system appear to preferentially affect actively transcribed genes in the brain, most notably dopamine receptors, amino acid transporters, and genes regulating synaptic transmission. Taken in conjunction with some estimates that there are upwards of 80 to 800 new L1 insertions per cell in some brain regions,[5] this represents a powerful mechanism by which these retrotransposons could modulate neurological activity and contribute to neurological decline during aging. Consistent with this idea, several neurological disorders, including Rett syndrome, Smith–Magenis syndrome, and schizophrenia are characterized by increased L1 activity.[6] Given the observations that L1 activity is upregulated in older brains, it is tempting to speculate that derepression of L1s may contribute to age-related pathologies of the nervous system, ranging from cancer to neurodegenerative disorders, particularly those associated with aging, such as dementias.

Cumulatively, two clear pictures of L1 biology are emerging. First, L1 elements are clearly active within somatic tissue. While they have historically been ignored in somatic contexts, the sheer volume of their activity necessitates reinvigorated research to investigate their biological importance. Second, L1 activity may be particularly important within the context of aging biology. Increased L1 activity is associated with aging in multiple tissues, and the discovery that SIRT6 links L1s to aging provides researchers with a platform for further inquiries into the relative contributions of L1 activity to aging phenotypes.

Interestingly, a number of therapeutic vectors exist that may allow researchers to attenuate L1 activity and perhaps delay the onset of L1-driven pathologies related to aging. For example, in our own experiments we found that overexpression of SIRT6 was sufficient to silence L1s and to restore L1 silencing in senescent cells. While overexpression of SIRT6 may not be tractable in a therapeutic context, SIRT6 activity can be increased by caloric restriction, reducing glucose consumption, or increasing NAD$^+$ bioavailability—interventions that have already shown promise in increasing longevity in animal models. (Such interventions are also showing promise in slowing the progress of some age-related neurodegenerative disorders. See "Nourishing the Aging Brain" on page 46.) Indeed, one study has indicated that caloric restriction is sufficient to repress age-related increases in L1 activity.[7] Another intriguing idea is the use of reverse transcriptase (RT) inhibitors. L1s cannot copy themselves without RT, and there are a number of powerful RT inhibitors on the market that appear to effectively abrogate L1 activity. Deciphering the extent to which L1s contribute to aging and how to stop them represents an exciting challenge for aging researchers in the coming years. ■

Michael Van Meter is a doctoral candidate in the research group of Vera Gorbunova and Andrei Seluanov, biology professors at the University of Rochester. They investigate the biology of aging and genome stability in long-lived animals such as naked mole rats, squirrels, and whales.

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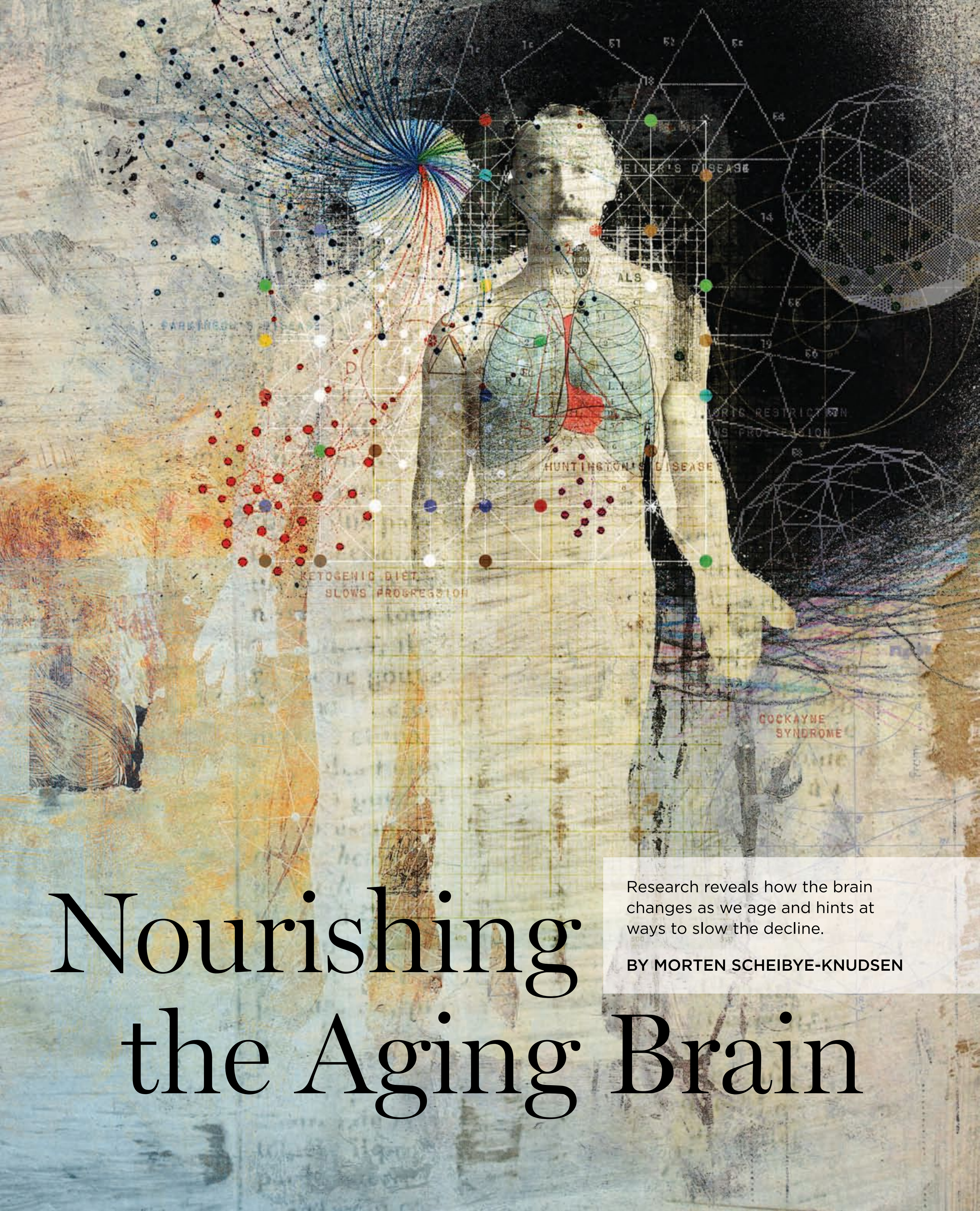

Nourishing the Aging Brain

Research reveals how the brain changes as we age and hints at ways to slow the decline.

BY MORTEN SCHEIBYE-KNUDSEN

The quest for immortality has inspired humankind since the dawn of civilization. Diverse ancient myths and religious stories tell of miracle drinks that bestowed eternal life on the gods, while kings across the ages have quested for comparable real-life elixirs. Chinese emperors, drawn by the promise of eternal life, swallowed concoctions containing everything from gold to arsenic and mercury, an often lethal cocktail. More recently, the late North Korean dictator Kim Il-Sung purportedly received transfusions of blood from young donors in hopes of extending his life. All these failed attempts at achieving longevity underscore the harsh truth that death will, inevitably, overtake us. Nevertheless, a century of research has brought us closer than ever to understanding what causes aging and what might be done to postpone its ultimate outcome.

One of the earliest successful attempts to extend life in the lab came from Cornell biochemist and nutritionist Clive McCay some 80 years ago, when he discovered that restricting the caloric intake of rats increased their life span by up to 50 percent. This observation has since been reproduced in organisms as distantly related as yeast, *Drosophila*, and *Caenorhabditis elegans*. But despite a wealth of research into why caloric restriction extends life, we are still rather far from pinpointing the mechanism behind the longevity effect of this dietary intervention. Of significant interest is how diets may affect aging in the brain, which is particularly sensitive to alterations in energy availability. Caloric restriction attenuates the progression of Alzheimer's disease in mouse models, for example, while diet-induced obesity exacerbates symptoms. Conversely, recent findings from my own lab suggest that accelerated brain aging, as seen in the premature-aging disease Cockayne syndrome, may be slowed through increased caloric and fat intake, while caloric restriction accelerates disease progression.[1]

Restricting the caloric intake of rats increases their life span by up to 50 percent, an observation that has been reproduced in organisms as distantly related as yeast, *Drosophila*, and *Caenorhabditis elegans*.

Clearly, caloric restriction is not universally beneficial. But by studying the influence of diet on aging in the brain, researchers have discovered a number of bioenergetic molecules and druggable targets that may serve as candidates for interventions to delay the onset of neurodegenerative disorders. Thus, while immortality remains a fantasy, living a longer healthy life may be an achievable goal.

Caloric restriction and health

Of the many benefits of caloric restriction, improved ability to maintain glucose homeostasis is probably the best established. Caloric restriction improves glucose tolerance, decreases insulin secretion from the pancreas, and increases insulin sensitivity in the peripheral tissues in model organisms as well as in humans. Not surprisingly, mice and humans on caloric restriction are also leaner, with reduced fat accumulation in the liver, resulting in a decreased risk of developing fatty liver disease. Markers of inflammation also decrease, and cardiovascular parameters such as blood pressure improve—effects that can also be explained by improved insulin signaling and glucose homeostasis.

In addition, calorie-restricted animals are smaller than their well-fed counterparts, perhaps corresponding to decreased cell proliferation, a phenomenon that occurs in response to energy deficits in both normal and cancer cells. Decreased cell proliferation may be important, since a cell can only undergo a finite number of divisions before it ceases to divide, a phenomenon called cellular senescence.

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In simple organisms such as yeast, two types of life span can be defined: a chronological life span, or the length of time a yeast cell can survive, and a replicative life span, or the number of cell divisions a yeast cell can undergo. Limited replicative potential is also observed in mammalian cells, where it is referred to as the Hayflick limit. (See "Of Cells and Limits" on page 54.) Decreased cell proliferation also leads to slower division of stem cells, allowing these progenitor cell populations to supply the various cell types of the body for longer periods of time. This sparing of stem-cell pools could explain why dietary restriction is particularly effective in maintaining tissue homeostasis in rapidly proliferating tissues such as skin, hair, and bone marrow.

Neural tissues, such as the brain and spinal cord, have a limited capacity to rejuvenate themselves through stem-cell renewal, however, perhaps explaining why dietary restriction may not impact these areas of the body as much as others. To complicate matters, rodents and many other model organisms do not normally suffer from neurodegeneration, and a possible neuroprotective effect of caloric restriction therefore cannot be extrapolated from longevity studies interrogating dietary interventions in those models. In order to circumvent this problem, researchers are studying neurodegenerative and premature aging diseases in humans to understand the effect of diet on brain health and aging.

Diet and disease

University of California, Los Angeles, researcher Roy Walford, a pioneer in the field of caloric restriction, felt so strongly about the benefits of this dietary intervention that he even practiced a version of it himself, convinced that it would allow him to live to age 120. Nevertheless, he was diagnosed with amyotrophic lateral sclerosis (ALS), a rapidly progressing neurodegenerative disorder, and died in 2004 at age 79. It is now relatively well established that caloric restriction exacerbates the progression of ALS, while increasing caloric intake attenuates the disease. This may reflect an increase in basal energy consumption in ALS patients.[2] Indeed, a recent study suggests that ALS patients can ingest a high-calorie diet without developing diseases such as obesity and diabetes.[3] Unfortunately, the increased catabolism, in combination with progressive feeding difficulties, leads to significant weight loss in ALS patients.

Age-associated disorders such as Alzheimer's, Parkinson's, Huntington's, and ALS appear to be influenced by dietary energetic intake.

These observations suggest that caloric intake can profoundly alter neurological health, supporting the idea that the human brain may be particularly sensitive to alterations in energy homeostasis. This sensitivity may stem from the organ's remarkably high metabolism: under resting conditions, the brain consumes roughly 20 percent of total body energy production, despite constituting only a minute fraction of body weight. In addition, the brain does not store energy as glycogen or fats as other organs do. In fact, the nervous system is largely unable to metabolize fatty acids for energy. Thus, the brain is highly dependent on glucose as a fuel source. Likely due to this strong glucose requirement for normal brain function, the majority of glucose uptake in the brain is insulin independent. Insulin increases brain glucose uptake by 10 to 20 percent, however, and central insulin resistance, which may occur in type-2 diabetes, decreases this insulin-stimulated glucose uptake.

As an alternative to glucose, the brain can draw energy from ketones, a group of metabolites synthesized from fatty acids in the liver. Interestingly, the addition of ketones as an alternative fuel source for the brain attenuates the progression of Alzheimer's in a mouse model of the disease.[4] In contrast, fatty-acid ingestion is associated with the development of the disease, though likely only in carriers of the *APOE4* risk allele. The mechanism for this is not well understood but may involve the role of APOE4 in the transport of cholesterol in the blood. Increased ingestion of fat leads to increases in circulating cholesterol levels that may predispose individuals with APOE4 to atherosclerosis, which in the brain vasculature is an independent risk factor for the development of Alzheimer's. Notably, diabetes, which leads to widespread atherosclerosis, is also an Alzheimer's risk factor.

Although this is speculative, one could imagine a decrease in neuronal energetic availability, due to insulin resistance and atherosclerosis of the brain vasculature, being involved in the pathogenesis of Alzheimer's disease. Similarly, a failure to meet the neuronal energy requirement in ALS may lead to loss of motor neurons. Further evidence for the role of dysregulated energy homeostasis in the body's neurons comes from Huntington's disease, a neurodegenerative disorder characterized by loss of voluntary movements, dementia, and often psychiatric symptoms. Like ALS patients, those suffering from Huntington's commonly have increased basal metabolism and unexplained weight loss. Interestingly, feeding a ketogenic diet slows disease progression in mouse models of both ALS and Huntington's diseases.[5,6] And in Parkinson's patients, who also tend to show increased energy expenditure and weight loss, a ketogenic diet has likewise shown promising results.[7]

It is clear that certain age-associated disorders such as Alzheimer's, Parkinson's, Huntington's, and ALS appear to be influenced by dietary energy intake.[8] (See illustration on opposite page.) Nevertheless, little is known about how normal brain aging is modulated by dietary changes. Because many model systems, including rodents, do not naturally develop neurodegeneration, this has been a particularly challenging problem to address.

ALS:

- ⬆ Increased basal metabolism
- ⬆ Caloric restriction exacerbates progression
- ⬇ Ketogenic diet slows progression

ALZHEIMER'S DISEASE:

- ⬇ Normal or lowered basal metabolism
- ⬇ Caloric restriction slows progression
- ⬇ Ketogenic diet slows progression

PARKINSON'S DISEASE:

- ⬆ Increased basal metabolism
- ⬇ Caloric restriction slows progression
- ⬇ Ketogenic diet slows progression

HUNTINGTON'S DISEASE:

- ⬆ Increased basal metabolism
- ⬇ Caloric restriction may slow progression
- ⬇ Ketogenic diet slows progression

COCKAYNE SYNDROME:

- ⬆ Increased basal metabolism
- ⬆ Caloric restriction exacerbates progression
- ⬇ Ketogenic diet slows progression

BRAIN DRAIN

The brain has a remarkably high metabolism, consuming some 20 percent of the total body energy under resting conditions. Largely unable to metabolize fatty acids for energy, the brain depends on glucose and secondary metabolites synthesized from fatty acids called ketones. Studies of several neurological disorders, which tend to be associated with increased basal metabolism, suggest that disease progression can be influenced by switching the energy source from glucose to ketones.

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Premature aging

Another window into normal brain aging could be disorders characterized by premature age-related changes in the brain. Cockayne syndrome patients, for example, develop very severe neurodegeneration—in addition to a host of other premature aging symptoms and extreme weight loss—and usually die by their teenage years.[9] The disease is exceedingly rare and is, in most cases, caused by mutations in the CSA or CSB genes, which encode two proteins involved in DNA repair. CSA and CSB proteins may also be involved in the control of transcription and in remodeling proteins that bind and package DNA, such as histones. Still, there is no good mechanistic explanation for the pathogenesis of this disease.

One intriguing aspect of Cockayne syndrome is how similar its clinical presentation is to known primary mitochondrial disorder symptoms, including progressive atrophy of the brain, lactate accumulation, hearing loss, and seizures. Since mitochondrial dysfunction has long been suspected to be a hallmark of the aging process, a defect in this crucial organelle's function could explain the accelerated aging features in Cockayne syndrome. Indeed, mitochondrial dysfunction has been observed in cells from Cockayne patients as well as in mice that harbor a truncation mutation in the murine homologue of human *CSB*. Like Cockayne syndrome patients, these mice show increased basal metabolism and loss of fat deposits. Feeding these animals a high-fat diet, however, rescues them from the disease phenotype, while caloric restriction exacerbates features of accelerated aging.

Because the mammalian brain does not metabolize fatty acids, a secondary metabolite, such as ketones, could be responsible for the beneficial effect of the high-fat diet. Indeed, ketones increase the life span of a short-lived nematode model of Cockayne syndrome. Ketones also ameliorate mitochondrial changes in models of Cockayne syndrome and have recently been shown to have life span-extending properties in wild-type *C. elegans*. Interestingly, ketogenesis, the production of ketones as a result of fatty acid breakdown, can be induced by fasting, suggesting that ketones could play a central role in the increased longevity effects of caloric restriction observed in the lab. Fasting, however, also leads to a decrease in blood glucose levels, perhaps leading to brain energy shortage in diseases such as Cockayne syndrome, where energy demand is increased.

The observation that ketones may slow aging caused by defects in DNA repair also suggests a link between genome instability and metabolic alterations. Levels of lactate, formed from pyruvate during glycolysis, are increased in the brain of Cockayne syndrome patients as well as in aged individuals. One factor influencing these levels is the ratio of NAD^+ to NADH, cofactors in the conversion of pyruvate to lactate.

Although a wealth of knowledge regarding interventions in specific age-associated diseases such as Alzheimer's and Parkinson's diseases have been reported, little is known about interventions that may modulate normal brain aging.

NAD^+ is a particularly interesting compound in the context of DNA repair. In the process of DNA-damage signaling, this molecule is consumed by the enzyme poly-ADP-ribose polymerase 1 (PARP1), which has previously been shown to be increasingly activated with age. Indeed, PARP1 is hyperactivated in several accelerated aging disorders thought to be caused by defects in DNA repair and concomitant accumulation of DNA damage. Greater PARP1 activity uses up more NAD^+, leading to a decrease in the NAD^+/NADH ratio and increased shunting of glucose to lactate. This also results in decreased production of acetyl-CoA, an alternative outcome of glycolysis and the fuel source for the mitochondrial citric acid cycle. (See illustration on opposite page.) But cells can also get acetyl-CoA by metabolizing ketones and fatty acids, and it is likely that the beneficial effects of a high-fat diet in Cockayne syndrome mouse models can be attributed to the normalization of acetyl-CoA levels.

In addition to being an important redox factor and DNA-repair metabolite, NAD^+ is a required molecule for the function of enzymes known as sirtuins, coded by a group of longevity genes, the activation of which may be central in the life-extending effects of caloric restriction in nematodes and flies.[10] These sirtuin enzymes metabolize NAD^+ to drive the deacetylation of various proteins such as histones and transcription factors. As a result of age-related decline in NAD^+, the activity of the mammalian sirtuin SIRT1 appears to be decreased. Interestingly, reconstitution of NAD^+ from precursor molecules can rescue such age-associated phenotypes, and in mouse skeletal muscle this effect may depend on SIRT1.[11]

Recent data suggest that the life-extending effects of ketones and NAD^+ both converge on the activation of SIRT1.[1] The role of SIRT1 in these processes may be of particular interest given that SIRT1 is highly expressed in the brain and represents a druggable target with multiple specific pharmacological activators currently being developed.[12] Indeed, several of these activators appear to be able to extend the life span of rodents. The efficacy of these compounds in ameliorating brain aging will be a particularly exciting aspect to investigate in future studies.

In the hunt for youth

Although a wealth of knowledge regarding interventions in specific age-associated diseases such as Alzheimer's and Parkinson's diseases have been reported, little is known about interventions that may modulate normal brain aging. We are, however, getting closer to an understanding of fundamental aspects of neurological aging, and we may soon be able to intervene in the aging process as a whole, perhaps with the benefit of preventing Alzheimer's, Parkinson's, and other age-associated neurological diseases. Increasing the level of circulating ketones, through dietary interventions or exogenous ketone sources, may be one relatively easy way to intervene, and could be efficacious either alone or in combination with other targeted interventions. Further studies are needed to understand the optimal dietary regimens and supplements, but there are grounds for optimism. ■

Morten Scheibye-Knudsen is a research fellow at the National Institute on Aging. His research has focused on brain aging and how various interventions might attenuate age-related neurological decline.

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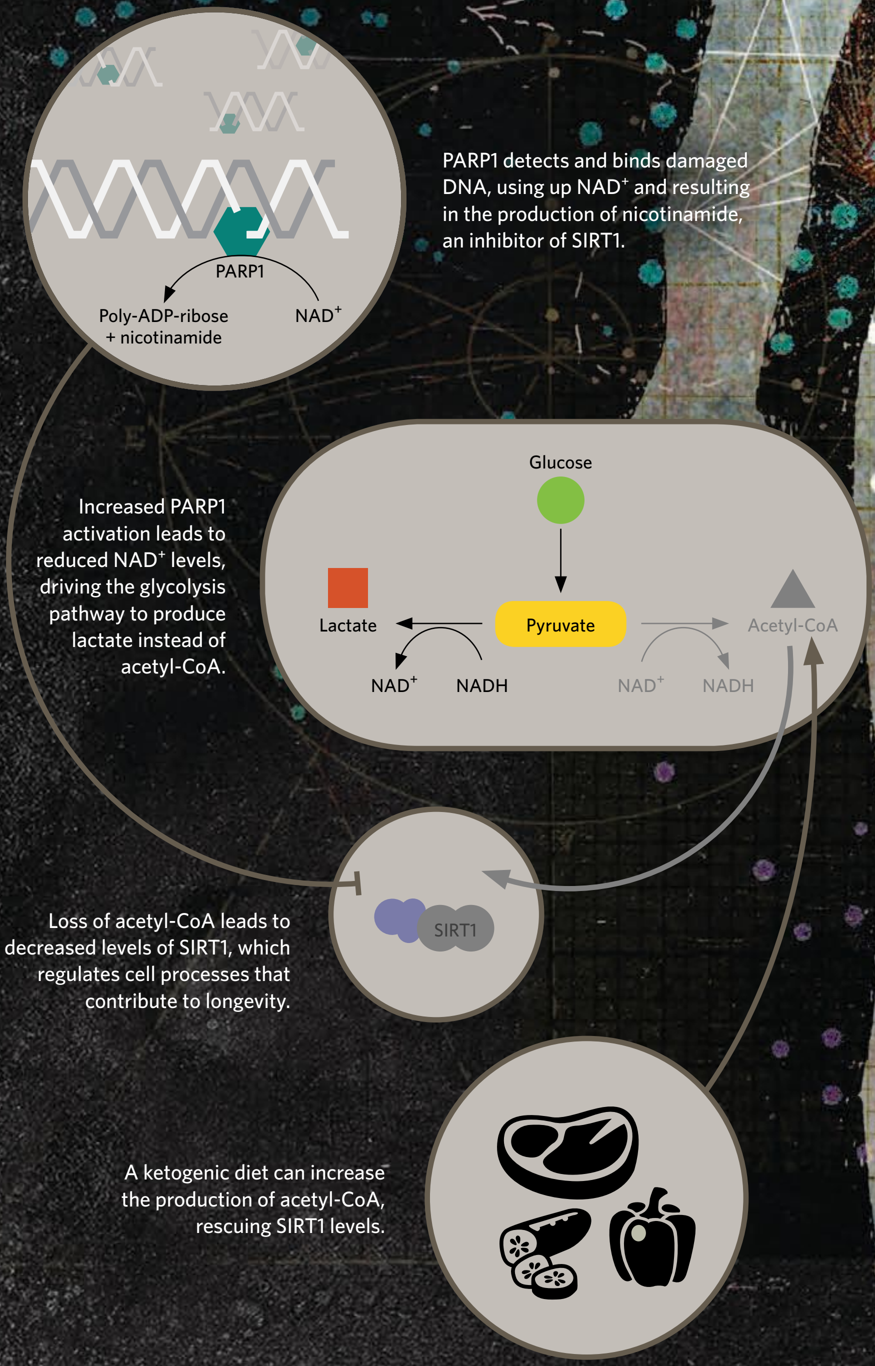
DNA DAMAGE IN AGING CELLS

A high-fat diet, and in particular a ketogenic diet, can rescue symptoms in animal models of Cockayne syndrome, an accelerated aging disorder caused by defects in DNA repair, suggesting that genome instability introduces metabolic alterations that occur in the aging process. Accumulation of damaged DNA leads to activation of poly-ADP-ribose polymerase 1 (PARP1). As a consequence, levels of NAD$^+$, a cofactor in glycolysis, and acetyl-CoA, an alternative to lactate as the outcome of glycolysis, decline, as does the activity of mammalian sirtuin SIRT1, which normally metabolizes NAD$^+$ to drive the deacetylation of proteins such as histones and transcription factors. Pharmacological inhibition of PARP1, restoration of NAD$^+$ levels, increases in ketone levels, and activation of SIRT1 have all shown promise in attenuating age-related decline.



PARP1 detects and binds damaged DNA, using up NAD$^+$ and resulting in the production of nicotinamide, an inhibitor of SIRT1.

Increased PARP1 activation leads to reduced NAD$^+$ levels, driving the glycolysis pathway to produce lactate instead of acetyl-CoA.

Loss of acetyl-CoA leads to decreased levels of SIRT1, which regulates cell processes that contribute to longevity.

A ketogenic diet can increase the production of acetyl-CoA, rescuing SIRT1 levels.

The Literature

GENETICS & GENOMICS

Rethinking Telomeres

THE PAPER

J.D. Robin et al., "Telomere position effect: regulation of gene expression with progressive telomere shortening over long distances," *Genes Dev*, 28:2464-76, 2014.

Telomeres are DNA repeats at the ends of chromosomes that protect genetic material from degradation. Because DNA polymerase cannot fully replicate the ends of chromosomes, telomeres shorten each time a cell divides. Telomeres also prevent the ends of chromosomes from fusing to one another by recruiting protective protein caps.

New work led by Jerry W. Shay and Woodring Wright of the University of Texas Southwestern Medical Center in Dallas demonstrates that telomeres are more than just buffer zones. The team found that as chromosomes fold within the nucleus, telomeres come into contact with faraway genes and alter their expression. As telomeres shorten, which happens with aging, chromosome looping and gene-expression patterns change.

"I'm delighted with this evidence that the [telomere] sequence may actually be doing some regulation and that the decrease of the sequence in some cells may drastically affect the way they are behaving," says Mary-Lou Pardue, who studies telomeres at MIT and was not involved in the research. She points out that telomeres are longer and have a more complex sequence than should be necessary to simply protect the chromosome ends.

Previous work had shown that genes near telomeres are repressed. Shay says that he and his colleagues began to suspect that telomeres were regulating more than just nearby genes when they found that the expression of a gene called *ISG15* increased as the telomeres of its chromosomes shortened, even while genes closer to the ends of the chromosomes remained unaffected.

Using a modified Hi-C technique, Shay and his colleagues mapped DNA looping in a 10-million-base-pair–long region of human chromosome 6. They found that, in cells with long telomeres, the telomeres interacted with multiple regions of the genome through intricate looping patterns. Imaging of specific genes using 3D-FISH revealed that, as the researchers manipulated telomere length, looping patterns reconfigured. Genes that had once been associated with telomeres were now relatively far away from them.

The researchers next used microarrays to analyze how telomere length affects gene expression across multiple chromosomes. They found that at least 144 genes within 10 million base pairs of a telomere sequence showed alterations in expression dependent on telomere length.

Finally, the researchers chose individual genes, including *ISG15*, whose expression is dependent on telomere length and assessed whether modifications in DNA folding accompany modifications in gene expression. Indeed, these genes' expression changed in concert both with telomere shortening and with looping-related changes in their telomere proximity.

The molecular mechanisms by which telomeres modulate gene expression remain unclear, as do the evolutionary reasons for telomeres' regulatory role. Shay speculates that telomere shortening can modulate gene expression so that it is appropriate for a cell's life stage. When telomeres become too short, cell division arrests, for instance. This allows the body to limit the number of times cells divide and avoid cancerous growth. Shay suggests that cells could alter gene expression to slow cell division in ailing cells even before telomeres become critically shortened. Or telomere shortening could trigger gene expression in healthy cells at pivotal times during development, such as during puberty or menopause. "The possibilities are endless and potentially very important," Shay says. **—Kate Yandell**

REGULATORY ROLE: Early in life, when telomeres (red) are long, chromosome looping brings them into contact with particular genes (green) ❶. As cells age, their telomeres shorten. Through mechanisms that are not yet understood, this alters chromosome looping and telomeres' interactions with genes, leading to age-related changes in gene expression ❷. Imaging using 3D-FISH (right panels) illustrates the distance between a certain gene and long (top) and short (bottom) telomeres.

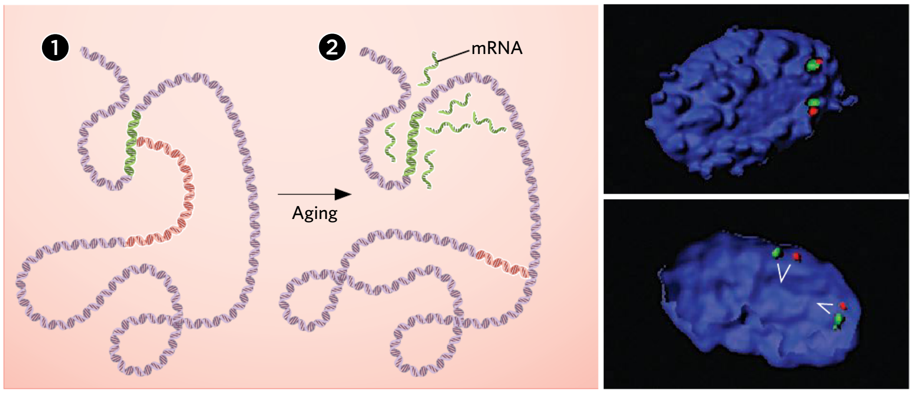
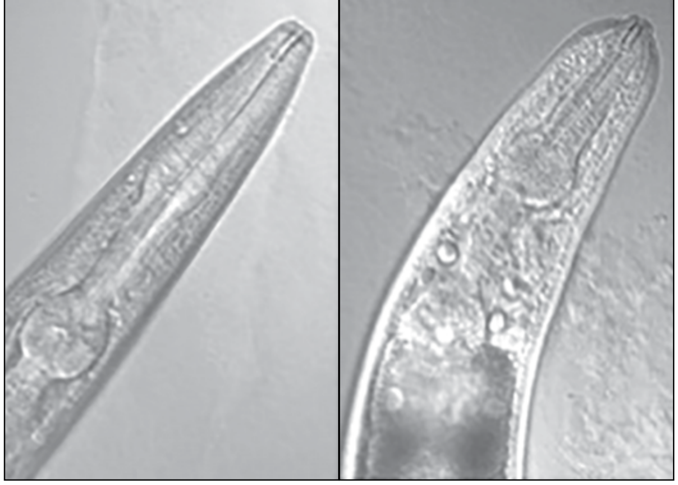


ILLUSTRATION © STEVE GRAEPEL; IMAGES COURTESY OF JERRY SHAY



AGING WORMS: Young (left) and old (right) specimens of the nematode *C. elegans*

MOLECULAR BIOLOGY

Long Live Collagen

THE PAPER

C.Y. Ewald et al., "Dauer-independent insulin/IGF-1-signalling implicates collagen remodelling in longevity," *Nature*, doi:10.1038/nature14021, 2014.

THE IMPETUS

One of the earliest observations about longevity in the roundworm *Caenorhabditis elegans*, a choice model organism for aging research, was that the worms live longer when the insulin/insulin-like growth factor 1 pathway is disrupted. This disruption normally sends worms into a hibernation-like state, called dauer, which increases life span by inserting a pause into the life cycle. Keith Blackwell of Boston's Joslin Diabetes Center and colleagues wondered if blocking the insulin/IGF1 pathway could increase life span even without this hiatus.

THE RESULTS

To this end, the researchers reduced the insulin/IGF1 pathway at temperatures that block entry into the dauer state, and the worms still lived longer. The team looked for changes in gene expression that accompanied this phenomenon and found the most striking upregulation among collagen genes. Such an uptick in expression was not unique to disabling insulin signaling; Blackwell's group found that collagens were also boosted in several other interventions that extended life span in *C. elegans*.

THE IMPLICATIONS

Collagens are most familiar as the proteins whose age-related dysfunction causes wrinkles. "This paper really suggests that collagens are not just, let's say, associated with external, . . . cosmetic usefulness, but overall improved health," says Malene Hansen of La Jolla's Sanford-Burnham Medical Research Institute. The study also suggests a new role in aging for the extracellular matrix, where collagens are found. "Mechanisms that will extend life [may] act by enhancing the function of the extracellular matrices and specifically of collagens," says Blackwell.

REMAINING WRINKLES

Whether collagen decline leads to aging or vice versa is an area for future research. "There's likely to be sort of a feedback loop" between the two, says Hansen.

—Ashley P. Taylor

OLD MICE: Ames dwarf mice (above, right) outlive wild-type mice (above, left), but their life span is unresponsive to changes in dietary methionine levels.

MOLECULAR BIOLOGY

Growth Hormone Guidance

THE PAPER

H.M. Brown-Borg et al., "Growth hormone signaling is necessary for lifespan extension by dietary methionine," *Aging Cell*, 13:1019-27, 2014.

THE PARADOX

Genetic defects in growth-hormone (GH) signaling extend life span in mice, as do diets minimizing the intake of a single amino acid, methionine. Yet scientists had previously observed that the Ames dwarf mouse, deficient in GH, lives longer but has upregulated methionine metabolism. Holly Brown-Borg of the University of North Dakota and her colleagues thus decided to examine how GH genetics and dietary methionine might interact to impact life span.

THE DIET

Brown-Borg's team compared four groups of mice on three different diets. Ames dwarf mice, GH-receptor knockout mice, transgenic mice that overexpressed GH, and wild-type controls were fed a diet with an 80 percent reduction, a 50 percent reduction, or a 50 percent increase in typical methionine levels. Methionine restriction extended the life span of the GH transgenic mice and the wild-type controls, but did not impact the life span of the Ames dwarf or GH receptor knockout mice.

THE RESISTANCE

"Animals that don't have growth-hormone signaling are unable to respond" to the drop in dietary methionine, says Brown-Borg. Follow-up studies by her group indicate that the altered metabolism of these GH-deficient mice is indifferent to changes in methionine intake. "They really don't discriminate either metabolically or in terms of life span when compared to other animals," she says.

THE MECHANISM

Matt Kaeberlein of the University of Washington praises the paper as a "really nice example" of a rare study investigating the interaction of genes and environment in aging, and hopes for a future mechanistic explanation. Brown-Borg speculates that the link might be epigenetic, given DNA-methylating enzymes' requirement for methionine.

—Jenny Rood

COURTESY OF KEITH BLACKWELL; CHUCK KIMMERLE AT UNIVERSITY OF NORTH DAKOTA

Of Cells and Limits

Leonard Hayflick has been unafraid to speak his mind, whether it is to upend a well-entrenched dogma or to challenge the federal government. At 86, he's nowhere near retirement.

BY ANNA AZVOLINSKY

Leonard Hayflick learned to culture cells under the tutelage of a famous expert, Charles Pomerat. In 1958, after completing his fellowship in Pomerat's lab, Hayflick became a member of the Wistar Institute in Philadelphia, providing cell cultures for its researchers. "It was a golden opportunity because it would leave me with plenty of time and resources to do what I wanted to do without worrying about getting grants or being subject to supervision."

Hayflick was interested in detecting viruses that might cause human cancer. He would extract viruses from the culture fluid in which he grew cancer cells and attempt to transform normal cells into tumor cells. Human adult cells frequently contained unwanted viruses, so Hayflick obtained tissue from fetuses to use as a source of virus-free normal cells. He found that tissue from the lung, which is already a discrete organ by three months of embryonic development, was best for making fibroblast cell strains. "Like everyone else who did cell culture, I was persuaded to believe the 60-year-old dogma that when you culture cells, they have the capacity to divide forever. In practice, this never happened and people attributed their failure to ignorance of culturing methods, media formulation, and other things like the cleanliness of glassware."

But Hayflick noticed a consistent pattern: in all 25 normal fetal cell strains he created, after about 50 population doublings, the cells were metabolically active but had stopped dividing. Together with cytogeneticist Paul Moorhead, he designed a simple but clever experiment to test whether the cells' limited capacity to replicate in culture was caused by microbial contamination or some unknown culture condition. It had recently been shown that female cells could be distinguished from male cells by the presence of Barr bodies formed from the inactive X chromosome in female cells. Hayflick mixed equal numbers of female cells that had divided 10 times with male cells that had doubled 40 times and found that after another 20 doublings of the mixed culture, only the female cells remained. "This showed that replication ceased not because of some extrinsic factor, but because of an intrinsic cell phenomenon—a revolutionary idea at the time."

"It is possible that the etiology of all age-associated diseases is in the molecular state that exists in old cells."

Hayflick's study was published in *Experimental Cell Research* in 1961, after first being rejected by another prominent journal—*The Journal of Experimental Medicine*. The rejection letter came from Francis Peyton Rous who received the Nobel Prize a few years later for his discovery of chicken tumor viruses. "I can still quote from that letter: 'Anyone who has worked with tissue culture knows that if the cells are provided with the proper milieu in vitro they will replicate indefinitely.' He also called my suggestion that our observation suggests something about cellular senescence and aging 'notably rash.'" The theory that all cells are generally immortal in culture was first postulated at the birth of cell culture in the early 1900s and was well publicized by Alexis Carrel of Rockefeller University in New York City who had developed a cell strain from chicken heart cells that he claimed had been growing for more than 40 years.

After showing that normal cells are mortal, Hayflick also reported, for the first time, that cancer cells were uniquely immortal—a claim that could not be made without first establishing that normal cells are mortal. He also discovered that his normal human fetal cells had a memory. When fetal cells frozen at different population doublings were thawed, the cells remembered the doubling at which they had been frozen and only divided until they reached a total of 50 divisions.

Hayflick's work was criticized and he was ridiculed. It took about 10 years for a more general acceptance that normal cells have a limited life span in vitro, a phenomenon now known as the Hayflick limit. The Nobel Prize–winning discovery of telomere shortening and the expression of telomerase explained Hayflick's observations.

Here, Hayflick talks about creating one of the most widely used cell strains, how he sued the federal government, and his discontent with the field of aging research.

HAYFLICK HEADS OUT

An explosive interest. Hayflick grew up in southwest Philadelphia and became interested in chemistry in elementary school, sparked by the gift of a Gilbert chemistry set. Hayflick made fireworks and rockets and experimented with heat and cold production. His mother encouraged him, even signing a permission statement that allowed Hayflick to purchase metallic sodium. With string, tin cans, water, and the sodium, he built rockets that traveled three to four stories into the air.

College-bound. After completing high school in 1945, Hayflick was accepted by the University of Pennsylvania, but first enlisted and served 18 months in the US Army so he could help pay for college through the GI Bill. "That was a fortunate decision. Had I not enlisted, I would have been drafted for the Korean War."

Building confidence. After majoring in microbiology at Penn, Hayflick worked for the Philadelphia-based pharmaceutical com-

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LEONARD HAYFLICK

Adjunct Professor of Anatomy
University of California, San Francisco

Greatest Hits

- Developed the first normal human diploid fibroblast cell strains such as WI-38 and the technology for producing vaccines against polio and other human viral diseases
- Discovered that normal human cells are mortal, have a memory located in the nucleus, and eventually become senescent. This finding is now called "the Hayflick limit" and is explained by telomere attrition.
- Discovered that cancer cells are immortal, now explained by the expression of the enzyme telomerase
- Discovered that "walking pneumonia" in humans is caused by a previously unknown species of mycoplasma
- Invented powdered cell-culture media and the first inverted microscope for viewing cell cultures
- Changed the thinking of biologists about their intellectual property rights

pany Merck, Sharp and Dohme and then returned to Penn as a graduate student. While earning a master's degree, Hayflick identified the first field isolate of a mycoplasma as the source of an ear infection in the rat colony at the adjoining Wistar Institute.

A new type of culture. Hayflick studied the behavior of mycoplasmas in cell culture before it was known that these organisms are common, but often silent, contaminants of cell cultures. (See "Out, Damned Mycoplasma!," *The Scientist,* December 2013.) "This was the first time that anyone purposefully put mycoplasmas into cell culture."

HAYFLICK HITS HIS STRIDE

A popular cell strain. Hayflick cultured the famous WI-38 strain from fetal female lung tissue in 1962 and developed the technology to make poliovirus and other vaccines in this strain. By then, Hayflick was getting requests for his cells from all around the world. WI-38 cells have been used to produce most of the world's human virus vaccines, including those against adenoviruses, measles, rabies, chicken pox, mumps, rubella, polio, and hepatitis A. Hayflick gave WI-38 gratis to the world's human virus vaccine manufacturers because, at that time, living cells could not be patented. But while Hayflick promoted the use of his cells for vaccine production, there was resistance, mainly from the National Cancer Institute and the precursor of the Food and Drug Administration's vaccine-control authority, which made unfounded statements that using Hayflick's cells would be dangerous because of their alleged potential to mutate and become cancerous.

Mycoplasma expertise. As head of Wistar's tissue-culture facility and an expert on mycoplasmas, colleagues would send Hayflick cell cultures to be tested for their presence. A visit from an NIH colleague got Hayflick working on "walking pneumonia," known medically as primary atypical pneumonia, a common disease in young adults worldwide. Previously, researchers had been unsuccessful in isolating a virus as the etiological agent for this form of pneumonia. From an egg yolk sample thought to contain the causative virus, Hayflick isolated a mycoplasma that had not been described before and named it *Mycoplasma pneumoniae*. This was the first human disease shown to be caused by mycoplasmas, and it made the front page of *The New York Times*.

Aging clock. After moving to Stanford University in 1968, Hayflick's lab continued to investigate how human cells in culture maintain a memory of the number of divisions they have made.

In 1975, he and his graduate student, Woodring Wright, showed that this counter was located in the nucleus.

A lawsuit. "My development of WI-38 was done without government or private support and because of the great demand for WI-38 cells, I was awarded an NIH contract only to 'store and distribute' the cells." When Hayflick moved to Stanford the contract ended, but the demand for WI-38 cultures continued. Hayflick began to charge $25—the same amount charged by the American Type Culture Collection at the time—to ship his cells, and put the funds into a holding account until a decision was made about disposition of those funds. "This seemed to me to be reasonable, but some people at the NIH and the FDA interpreted this to be theft of government property, and that I was selling WI-38 for personal gain. They entered my lab in my absence and confiscated all of the frozen WI-38 ampoules." Hayflick sued the federal government in 1975, claiming that the NIH had violated the Privacy Act of 1974 by releasing their findings before allowing him to reply and by illegally confiscating WI-38. "Who owns WI-38 or any other continuously dividing human cell culture is not easily settled. There are several stakeholders—the researchers who give the cells value, their institution, the individual from whom the cells are derived, and the organization that supports the research."

The suit was ultimately settled outside of court, at the request of the Justice Department, who found itself in an impossible position for a number of reasons. First, living cells were now patentable. Second, an executive order by President Reagan and the Bayh-Dole Act made it legal to use tax payer–supported research materials for commercial exploitation. Third, the emerging biotechnology industry was founded on materials developed in federally funded academic research labs. If the government prevailed in Hayflick's lawsuit, the biotech industry would be jeopardized, so industry lawyers prepared amicus briefs on Hayflick's behalf should his case go to trial. Finally, the NIH itself was by now offering products made in their own laboratories for commercial use.

As part of the settlement, Hayflick received some of the confiscated ampoules of WI-38 as well as the funds from mailing the WI-38 cultures. All of these funds went to pay his legal expenses.

And Hayflick had supporters. After the settlement, a letter published in *Science* signed by 85 scientists stated that they objected to the behavior of the NIH and the FDA (215:240-42, 1982). According to Hayflick, his position that biologists, like other scientists, have intellectual property rights helped start a revolution in thinking, resulting in many biologists later becoming successful entrepreneurs.

Due credit. Hayflick has been credited with starting the field of cellular biogerontology, the study of the aging of cells. His work set a new direction for aging research. "The focus had been on extracellular causes of aging such as stress or radiation. I suggested that what I had found was teaching us something about the biology of aging, and the term senescence became widely used. My work refocused the field onto intracellular events as the etiology of aging," he says.

HAYFLICK HAS A SAY

A matter of linguistics. "The term 'research on aging' has become muddled. Most scientists fail to understand that despite the fact that the 'aging' tag exists on their center, institute, or organization, most of the research is not on aging, but on age- associated diseases or longevity determinants. One problem that this linguistics issue creates is the diversion of more than 50 percent of the National Institute on Aging's budget to Alzheimer's disease research and less than 5 percent on the fundamental biology of aging. We should do Alzheimer's research, but not at the expense of research on the fundamental biology of aging. For more than 50 years, aging research has meant research on age-associated diseases and this will tell us nothing about the cause of aging. It is remarkable to observe that a major medical concept that 'the greatest risk factor for all age-associated diseases is aging' has resulted in so little research on aging. It is possible that the etiology of all age-associated diseases is in the molecular state that exists in old cells or cells near the end of their lineage. I've been vocal about this for 40 years. And as a consequence of that you don't make too many friends. But I am more interested in the truth than making friends."

Longevity vs. aging. "Many confuse aging with longevity determination, which is indirectly driven by genes. There are no genes for aging. There are genes to maintain the biological functionality of an organism up to and including reproductive success. There is no mechanism that immediately causes death after reproductive maturation; it's too costly. Longevity determination asks 'Why do we live as long as we do?' Aging research asks 'Why does everything ultimately fail?' and that is a huge difference."

Just the facts. Rebecca Skloot, author of *The Immortal Life of Henrietta Lacks,* had contacted Hayflick during her research for the book as he knew the personalities involved, including those who had originally cultured the HeLa cells. "Unfortunately, much of what I told her was hyped by her editors, as I'll give her the benefit of the doubt. There are a lot of inaccuracies in the book. One is the allegation that HeLa cells have saved lives and benefited the health of more people than any other cell population. That is false. WI-38 has benefited 2 billion people who have received vaccines made in these cells. Another is that HeLa cells were the first immortal cell population; that was L929 developed by Wilton Earle at the NIH nine years earlier. But this doesn't take away from importance of the book. It raises the property rights and ownership issue of self-duplicating biological entities, which I have been addressing for 30 years."

Retirement? Hayflick continues to mentor, consult, write, lecture, organize conferences on aging, and travel to meetings. "I have not stopped doing anything except running a wet lab. I've never retired because I don't know a definition of that word that applies. I have an enormous correspondence network worldwide, and I spend a lot of time keeping up with the scientific literature. I am also writing my autobiography. My mother lived to be 106, so I am hopeful that I may have inherited some of her longevity determinants." ■

Weiwei Dang: Epigenetics in Aging

Assistant Professor, Huffington Center On Aging, Baylor College of Medicine. Age: 38

BY JEF AKST

Weiwei Dang attended Peking University in his hometown of Beijing, China. Having taken a liking to science in high school, he spent a year working in the lab of molecular biologist Bing-Gen Ru developing a method to test the concentration of metallothionein in rabbits as an indicator of environmental levels of heavy metals. By the time he graduated in 1999, he knew he wanted to pursue a career in the lab, and "at the time, it was everybody's opinion that the U.S. is at the very forefront of biomedical research." So he headed to Southern Illinois University in Carbondale, and there he spent a graduate rotation in the group of biochemist Blaine Bartholomew, who introduced him to chromatin and epigenetics.

In Bartholomew's lab, Dang interrogated the interactions between the chromatin-remodeling enzyme ISW2 and nucleosomes. He mapped the DNA locations on the nucleosomes where the enzyme made contact; then, he mapped those contact positions on the enzyme.[1,2] "He did some pretty hard work, to be honest," says Bartholomew, now at the University of Texas MD Anderson Cancer Center, adding that the DNA photoaffinity approach Dang used is a tedious process that requires patience and attention to detail. "It does take someone with good hands and good technique."

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Dang was hooked on chromatin biology. As he wrapped up his PhD work, he began scoping out potential postdoc advisors at meetings, and was fascinated by a presentation given by Shelley Berger, now director of the Epigenetics Program at the University of Pennsylvania's Perelman School of Medicine. In March 2006, Dang joined Berger's lab, then at Philadelphia's Wistar Institute, where, among other projects, he launched into studying the role of epigenetics in aging. Dang purified young and old yeast cells, then applied available antibodies for assaying histone modification to test for changes that occurred during aging. Among the differences he and his colleagues found was increased acetylation of the lysine-16 (K16) residue on the tail of histone H4.

Looking more closely, he and his colleagues found that the enzyme Sir2, a known aging regulator in yeast, deacetylates K16, and that Sir2 levels decrease in older yeast cells. Meanwhile, acetylation increases in the subtelomere region, between the telomeric caps themselves and the chromatin, which correlated with fewer nucleosomes.[3] "Basically, what it suggests is the more acetylation you have, the more loose the chromatin fiber becomes," Dang says. Finally, he found that altering K16 acetylation can affect life span: less acetylation leads to longer-lived yeast. "I think [the study] has had a really big impact on the [aging] field," Berger says.

Now in his own lab at Baylor College of Medicine, Dang continues to study the role of Sir2 and other epigenetic factors in aging. His group is also conducting screens for overexpressed genes that affect life span, using a high-throughput method Dang mastered and streamlined in Berger's lab. His lab is also moving into mammalian model systems, hoping to apply what his group learns in yeast to mammalian aging using mesenchymal stem cells.

"In science, it's the ability to look small and look big—to be able to focus on specific mechanisms and specific questions, but being able to ask big questions about those, being able to formulate important hypotheses," Berger says. "He has the ability to do that. I think he's really going to make an important mark as an independent scientist." ■

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Chill GPS

Keeping track of frozen biological samples

BY JEFFREY M. PERKEL

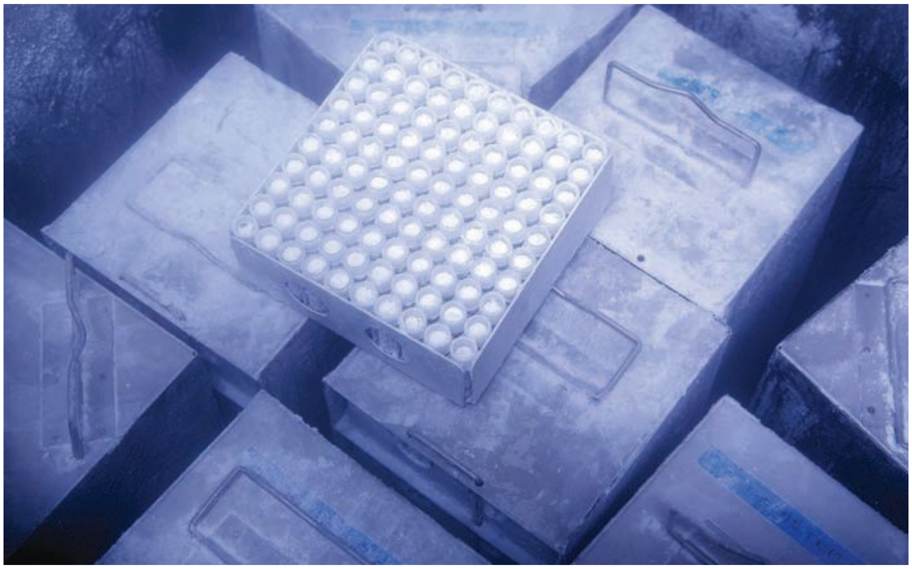
Say you're heading out of town on vacation. You and your colleagues have been trying to assemble some new genetic constructs, but so far all you've got is a set of purified inserts, PCR products, and half-finished plasmids. As you run out the door to catch your flight, you hurriedly place them all in a box in the −20 °C freezer, without logging their location in your notebook.

Your colleagues want to continue the work, but they don't know where to look for the samples. And you? You're off the grid, scuba diving in the Turks and Caicos.

It's a truism of today's life-science laboratories that their most valuable assets are typically housed either in identical polypropylene Eppendorf tubes in the freezer, or in screw-cap vials under liquid nitrogen. There's little room to write on those tubes, and adhesive labels can come loose. Even if the labels stick, some people have appalling penmanship. And even the neatest handwriting can only convey so much. How, after all, is anyone to remember, several years and two postdocs later, what is meant by a cryptic string of letters and numbers like SHC1-1? Is that a plasmid or a primer? Full length or partial? And what's its concentration? In such a situation, researchers typically have little choice but to discard the vial, another casualty of poor sample management.

The problem is especially acute in large labs with a steady turnover of grad students and postdocs, but even modest operations are not immune. Samples get moved, boxes misplaced, and labels smudged. Projects may get put on the back burner, only to be resurrected years later. Good documentation of exactly which sample is where is key to successfully bringing such projects back to life.

The Scientist asked four laboratories about the strategies they use to keep their precious samples in order. This is what we learned.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FACILITY: The George Daley laboratory, Boston Children's Hospital

PROBLEM: Daley's lab has from 20 to 40 lab workers at any one time, sharing three −80 °C freezers and two liquid-nitrogen tanks. They generate a lot of reagent vials, offering boundless opportunities for samples to get misplaced or lost.

SAMPLE-TRACKING SOLUTION: Daley is a pioneer of human embryonic and induced pluripotent stem cell (iPSC) research. His team has developed countless cell lines and reagents over the years, many of interest to the wider scientific community.

When lab manager Beth Kaleta joined in 2007, the only clue to navigating that collection was a binder with "a lot of chicken-scratch" detailing what reagents were where. So, she started an Excel spreadsheet to keep track of everything—that is, plasmid A is located in freezer 3, shelf 2, box 4, spot D2. Every researcher since 2007 has had their samples logged in this fashion, Kaleta says. "It's the older stuff that's hard to inventory," as it isn't always possible to match a tube with a lab notebook entry. The lab is also considering a sample barcoding strategy, though no decision has yet been made.

Every postdoc in the lab is guaranteed at least one full rack in liquid nitrogen (five boxes, 81 samples per box) and one rack (16 boxes) in the ultralow-temp freezer. While in the lab, researchers are in charge of their own material. That can present a problem if a postdoc leaves suddenly, as there may be insufficient documentation of what he was working on and where he kept it. "One person just up and left the lab, and I had no idea what her stuff was," Kaleta says.

When someone departs, Kaleta performs an exit interview in which she and a senior scientist meet with the researcher to ensure that critical reagents and cell lines are accounted for. Only some materials are retained, she notes: Key cell lines are passed to the Harvard Stem Cell Institute's core laboratory for expansion and distribution, freeing the lab from getting overwhelmed by requests. Important plasmids are stored and/or transferred to Addgene, a nonprofit biological reagent repository and distributor, which currently has 75 Daley lab plasmids available among more than 33,000 deposited by 2,000 labs from all over the world. (See "Sharing Made Easy," *The Scientist*, September 2012.) Generally, the lab retains two vials of each reagent for itself. "That usually frees up a decent amount of space," Kaleta says.

According to Kaleta, transitioning from binder to spreadsheet has minimized the number of times the lab comes across reagents they simply cannot identify. But it still happens. One former postdoc, she says, designed multiple iPSC lines and named them using a coding strategy only the postdoc knew—and has since forgotten. "We had to throw some things away."

FACILITY: The Tony Pawson laboratory, Lunenfeld-Tanenbaum Research Institute (LTRI), Mount Sinai Hospital, Toronto, Ontario

PROBLEM: Starting in 2005 the Pawson lab, in collaboration with other scientists at LTRI, began accumulating clones from the US National Institutes of Health's human and mouse Mammalian Gene Collection (MGC) libraries. As they acquired what became tens of thousands of bacterial stocks, they needed a way to keep track.

SAMPLE-TRACKING SOLUTION: LTRI's MGC clone collection was purchased in fits and starts, says staff scientist Karen Colwill—a few thousand here, a couple thousand there. Those clones were shipped in microtiter plates, and that's how they are stored today. The collection comprises 70,791 clones arrayed in 751 96- and 384-well plates stored in a single −80 °C freezer, with 25 racks and 66 plates per rack. Naturally, finding any one clone requires solid bookkeeping, as does locating any of the institute's other reagents, including nearly 1,000 cell lines stored in liquid nitrogen. Colwill and lab software engineer Marina Olhovsky codeveloped OpenFreezer (openfreezer.org) to make that possible (*Nat Methods*, 8:612-13, 2011).

Designed for permanent and secure reagent archiving and simultaneous multiuser access, OpenFreezer is a free, open-source, Linux-based Web application. "It's not a simple download onto a Windows machine," Colwill says. "You need to have someone with some reasonable understanding of programming" to get it up and running. Still, she says, it is "reasonably user-friendly" and "intuitive," and more than 20 institutions use the application.

Users can customize OpenFreezer to track and inventory any type of reagent, but by default it can handle plasmids, inserts, oligonucleotides, and cell lines. The database also captures associated variables, such as nucleic-acid sequence, sample concentration and volume, and, of course, storage location. Users interact with the system via a basic Web browser, and a mobile app is in development, Colwill says. A new release, anticipated sometime in 2015, will include modules to add protocols and track lab orders.

OpenFreezer allows users to search for samples by filters such as keywords and database identifiers, and to identify reagents based on physical location. According to Colwill, the Lunenfeld OpenFreezer database currently tracks some 186,000 samples representing multiple labs and users. Those users are responsible for ensuring that the database remains up-to-date, of course—the system falls apart if researchers store samples without logging them in and recording their vital stats. Still, she says, it should give lab managers greater confidence in using and distributing key reagents, as labs are obligated by convention to send published reagents to other researchers upon request. "It really makes it that much easier to reuse a clone generated by a former lab member when it's that well documented."

FACILITY: Johns Hopkins Biological Repository

PROBLEM: The Johns Hopkins Biological Repository (JHBR) occupies 8,000 square feet of lab and storage space split between two locations. It houses more than 2.5 million biological specimens collected over 30 years—everything from blood serum and nucleic acids to toenail clippings and hair—stored in 62 vapor-phase liquid-nitrogen tanks. Being able to retrieve samples on demand is of paramount importance.

SAMPLE-TRACKING SOLUTION: In 1997, Stacey Cayetano, clinical laboratory manager for the JHBR, began investigating the phasing-out of handwritten labels in favor of cryogen-safe sample barcoding to have "a more seamless transfer of data." The software system she selected was Freezerworks (freezerworks.com) from Dataworks Development, Inc.

At the time, Cayetano explains, Freezerworks was the most user-friendly solu-

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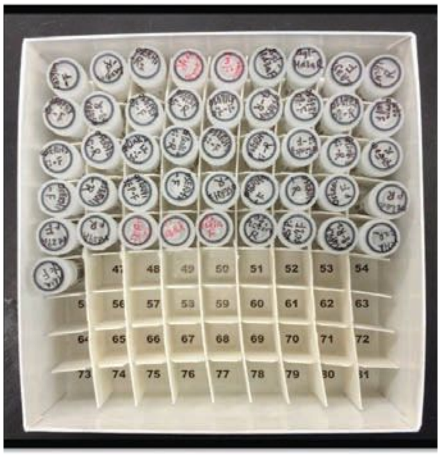
BEFORE AND AFTER: Unordered primer samples (left) made searching for specific primers difficult. Using an indexed-based system with location number and box name (right) allows for easy retrieval.



tion she could find, and also the most configurable. Among other things, JHBR stores patient samples for human clinical trials, and the information it captures for each one varies with the specific research protocol in the trial. "At any given time we are juggling 30 to 40 research protocols, so we needed flexibility," Cayetano says.

Freezerworks is a relational database that links samples and sample properties to their locations. JHBR stores anonymized info on what a sample is, how it was processed, and its volume. Users can execute fairly granular searches, Cayetano says. For instance, given a list of patients that meet certain demographic requirements (JHBR does not store such data, for privacy reasons), users can then request that JHBR pull, say, all HIV-negative sera of more than 500 µL that have never been thawed. "Freezerworks would give us the subset of samples that meet that criteria," she says.

Like OpenFreezer, Freezerworks uses a client-server architecture. The Johns Hopkins School of Public Health maintains the server, Cayetano says, and JHBR employees access it on workstations. There's also a Web interface, so remote users with appropriate permissions (such as research clients) can search their samples in a read-only mode, then send pull requests to JHBR staff for processing.

The system, she says, is simple to use—"I've probably taught 75 or more techs how to use the software, easily"—and Dataworks Development has been responsive to addressing bugs and feature requests, she adds. Cayetano budgets about $5,000 a year to maintain a service contract for the software, which gets her maintenance upgrades gratis, but major upgrades are extra. "I've been using [the software] for so long, it's a standing line item in my projections," she says.

But should the company stop supporting the software, JHBR won't be stuck with millions of records it can no longer access; Cayetano could simply export the data into a flat file format and import it elsewhere, she says.

FACILITY: The Shunyuan Xiao laboratory, University of Maryland

PROBLEM: The Xiao lab has more than 2,700 DNA oligonucleotides in its collection, whose specifics and locations were tracked on different Excel spreadsheets. Keeping those spreadsheets standardized and up-to-date was well-nigh impossible, and finding a particular reagent (and its sequence) could take 30 minutes or more. "It was no way to live," says Harley King, a grad student in the lab.

SAMPLE-TRACKING SOLUTION: As the de facto lab manager, King began investigating options to reorganize the Xiao lab reagents. He settled on Quartzy (quartzy.com).

Quartzy is a free, cloud-based lab management system introduced in 2009. Among other things, it allows users to upload reagent storage info in the form of Excel spreadsheets. A high school intern spent a few weeks organizing the lab's collection, moving reagents from disordered boxes into boxes that were numbered, and logging the info as he went. "Once we were on board, we've never looked back," King says.

In addition to inventory management, Quartzy also facilitates reagent ordering, document storage (for instance, standard operating procedures), and instrument scheduling. Users can search by location or content, and can submit purchase requests directly to the lab manager. The software even suggests alternative pricing for needed reagents—indeed, Quartzy has partnered with life-science tools vendors to provide these suggestions, which explains its pricing model. "The other day, they saved me $12 on agarose," King says.

Jerrold Turner, a professor of pathology at the University of Chicago, also uses Quartzy, but only for instrument scheduling and order tracking; freezers he organizes with spreadsheets. Quartzy, he says, would be "great," but "like all solutions I've seen, it falls down at the human level," explaining that it's difficult to reliably keep such databases up to date. "Organizing people in a lab can be like herding cats," Turner says.

Turner does keep his mouse colonies and liquid nitrogen tanks organized with software, however. He uses a customized FileMaker Pro database to track the liquid nitrogen stocks, for instance, and ensures the database is current by having users go through a gatekeeper lab member to physically access the tanks themselves. For mice, his group began using an online solution called mLIMS last year. "Effective tracking has helped lab members think through their strategies more efficiently," Turner says. "Our per diem costs have been cut by 40 percent with no loss in productivity." ■

ABRAHAM IPE

Red Hot

CRISPR/Cas is all the rage—and getting more precise and efficient.

BY KELLY RAE CHI

Scientists have a fever for genome tinkering, and the latest thing shooting up temperatures is CRISPR. The number of publications referring to CRISPR/Cas technology has mushroomed since its first use as a genome-editing tool in 2012. In a testament to the method's popularity, a recent guest lecture at Vanderbilt University by CRISPR pioneer and 2015 Breakthrough Prize winner Jennifer Doudna packed a 300-person classroom and a 160-person overflow room—which then itself overflowed, recalls attendee Douglas Mortlock, a research assistant professor at Vanderbilt who blogs about advances in CRISPR technology.

CRISPR (clustered, regularly interspaced, short palindromic repeats) is named for particular DNA loci that are found in many archaea and bacteria. CRISPR works with associated nucleases, including Cas9, to protect the cells from viral infection by inserting short snippets of viral DNA into the CRISPR cassette. By combining the Cas9 nuclease with a short guide RNA that's custom-designed to bind a specific target, CRISPR/Cas can easily edit any gene you want. Just in the past year, for example, it has allowed researchers to cure a rare liver disease in mice, to excise HIV-inserted genes from human immune cells, and to block HIV from entering blood stem cells. CRISPR/Cas is easier than the other nuclease-based editing technologies, says John Schimenti of Cornell University; scientists are basically a reagent catalog and a round of PCR away from having everything they need to utilize CRISPR.

Anticipating the potential for the technology to cure human genetic diseases, researchers want to make the CRISPR/Cas9 system as efficient and specific as possible. Its chief drawback has been that, once inside the cell, it can dock (and cut) at undesired sites, potentially producing off-target edits that could be a deal breaker for clinical applications. Researchers are working on ways to avoid such unintended edits, including better tools for predicting edit sites in the first place. Scientists are also developing more-efficient ways of getting CRISPR elements inside cells, because only a small subset of cells takes up the system.

Last March, *The Scientist* published a newcomer's guide to CRISPR (see "A CRISPR Fore-Cas-t"). In the intervening year, researchers have ramped up efforts to improve the technique. Here are some of the methods they're exploring.

DESIGN BY ZENGRONG ZHU, KIHYUN LEE AND QING LI. ARTWORK BY TERRY HELMS.

A CUE FROM CANCER

Skewing DNA repair with small molecule SCR7

RESEARCHER: John Schimenti, professor of genetics, Cornell University

PROJECT: Using CRISPR/Cas9 to develop mouse models of cancer and meiosis

PROBLEM: CRISPR/Cas9 editing works by making breaks in the genome that are repaired via one of two pathways. The broken ends are glued back together in a process called nonhomologous end joining (NHEJ), which is efficient but error-

MUTATION MANAGEMENT: A single-celled mouse zygote is microinjected with the CRISPR/Cas9 system. Addition of SCR7, an anticancer drug, shortly after injection alters the ratio between two DNA repair pathways to reduce unintended mutations that can arise during the gene-editing process.

CHRIS ABRATTE

prone; or, if you supply a synthetic piece of DNA containing the precise desired mutation, it can be incorporated through homology-directed repair (HDR).

"The problem is that those two processes are in competition," says Schimenti. "The end joining [NHEJ] becomes a nuisance because way more of the experimental mice turn out to contain these unintended mutations due to the end joining." His group needed a way to tip the scales toward the HDR pathway to produce more-precise edits.

SOLUTION: For Schimenti's group, a clue about improving the HDR/NHEJ ratio came from past genome-editing studies of *Drosophila* embryos, in which researchers genetically inhibited a molecule (DNA ligase IV) involved in the NHEJ pathway. It so happened that a small-molecule inhibitor of that ligase, SCR7, was subsequently identified as a potential treatment for cancer; Schimenti's group decided to try SCR7 instead of genetic perturbation.

In a study of mouse embryos in which the group aimed to incorporate a two-base mutation into one gene, the addition of SCR7 improved the ratio of HDR:NHEJ from 1:10 to 1:2.5 (*Genetics*, 199:1-15, 2015).

GETTING STARTED: Simply add SCR7 to your media after microinjecting Cas9 and synthetic DNA into your single-celled zygote. Other groups, such as Douglas Mortlock's at Vanderbilt University, are also trying it. Schimenti's lab routinely adds SCR7 to their experiments because it doesn't seem to hurt, though "to see if it really helps for other genes, you'd have to try to make mice with your designed event, with and without the chemical," he says.

CONSIDERATIONS: Zygotes bathed in SCR7 (50 µM) showed better survival rates up until the two-cell stage, but prolonged culture of the embryos in the presence of SCR7 negatively affected their progression into blastocysts, for reasons that aren't clear. That shouldn't be a problem, Schimenti says, since you transfer them to the female mouse before then.

COST: SCR7 is available from Xcessbio, $129 for 2 mg.

INDUCIBLE CRISPR FOR HUMAN CELLS

Cas9 genetically built into human stem cells and expressed on demand

RESEARCHER: Danwei Huangfu, developmental biologist, Center for Stem Cell Biology, Memorial-Sloan Kettering Cancer Center

PROJECT: Using human pluripotent stem cells to understand the roles and interactions of specific genes in pancreatic development

PROBLEM: Among the challenges of using CRISPR is getting the system's components inside cells, which is usually done through electroporation. The process of inserting the system into human stem cells is especially inefficient; it takes months to create a single mutation, Huangfu says.

Huangfu's projects necessitated deleting both copies of a gene or multiple genes, which required multiple rounds of targeting. She needed a more efficient system.

SOLUTION: The most difficult step is to transfect the large Cas9 gene into cells, so Huangfu's group created a cell line with the Cas9 component already integrated. To do this, they employed a related genome-editing technology, TALEN, that uses DNA-binding domains rather than guide RNAs for targeting (*Cell Stem Cell*, 15:215-26, 2014). "Using TALENs, we integrate the Cas9 into a very specific location in human [embryonic stem] cells, which we know is not subject to silencing effects," Huangfu says.

The group also designed the cells so that they express Cas9 upon the addition of the drug doxycycline. The resulting approach, called iCRISPR, allows the scientists to turn on genome editing at different time points in the differentiation process. (iCRISPR is not to be confused with CRISPRi, which, similar to RNAi, reversibly blocks transcription, without cutting.)

iCRISPR cuts down the number of main steps in the protocol to one: transfecting the cells with guide RNA, which is smaller and easier to get into the cells. "We're surprised that [iCRISPR is] working so well," Huangfu says. Using the system, her team has been able to mutate 12 genes in a month's time.

GETTING STARTED: Huangfu's group has published a detailed protocol in *Methods in Enzymology* (546:215-50, 2014). Researchers who are already experienced with gene targeting in human stem cells can get the plasmids from Addgene and make iCas9-expressing cells themselves, she says. With all materials in hand, and the expertise, it takes one to two months to make the iCas9 cells. You should allow another month or two for making the genome-edited cell line.

Those not interested in making the iCas9 cells from scratch can contact the SKI Stem Cell Research Facility.

CONSIDERATIONS: Although Huangfu's group hasn't seen significant off-target effects, the team uses two strategies to overcome potential off-target effects, similar to those used in RNAi: rescue experiments and two different CRISPRs to target the same gene.

COST: Plasmid costs $65 through Addgene; iCas9 cells cost $600 for the first line and $400 for the second when purchased through SKI.

DELIVERING SUPERCHARGED PROTEINS

A bit of charge helps the CRISPR/Cas9 system slip in

RESEARCHER: David Liu, professor of chemistry and chemical biology, Harvard University

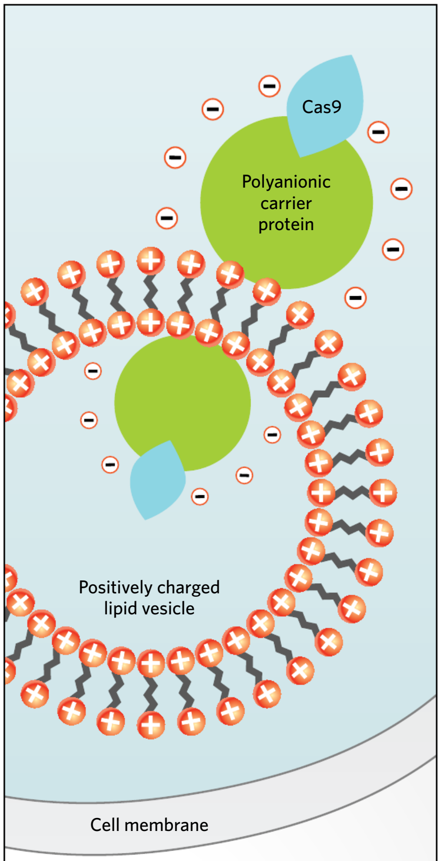
PROJECT: Developing ways to treat genetic deafness in a mouse model

PROBLEM: Genome-editing proteins need to get into the nucleus of target cells to do their job, but delivery of CRISPR/Cas9, as with all large molecules, is a major challenge.

And when the CRISPR/Cas9 system is delivered as a DNA plasmid, the Cas9 nuclease can work a little too well: it cuts the target gene, but hangs around and cuts some more, boosting the likelihood of off-target edits.

Liu and his group reasoned that delivering the protein directly would help solve the latter problem: the proteins could do their editing for a shorter, well-defined period of time and then degrade naturally. But Liu needed a way to get the proteins into cells.

SOLUTION: Work the group had done earlier suggested a potential strategy to deliver genome-editing proteins into mammalian cells: coat the proteins with negative charge. They then packed these negatively charged proteins into positively charged lipid-based detergents, which get swallowed by cells via endocytosis.



NEGATIVE HELPERS: Cas9 (turquoise) enters cells more easily when fused to a green fluorescent protein engineered to have multiple negative surface charges (green). Upon complexing with positively charged lipids, Cas9 enters the target cell via endocytosis.

THE SCIENTIST STAFF, BASED ON *NAT BIOTECHNOL*, 33:73-80, 2015

The protein delivery approach resulted in more-efficient genome modification and higher specificity for the targets, compared with traditional DNA transfection using a virus.

The fact that protein doesn't stick around for long helps explain the transient spike of genome-editing activity the researchers saw in the cells, Liu says. They were also able to deliver Cas9 in vivo, modifying hair cells in the mouse inner ear. (For a different method of transfecting hair cells, see "Tricky Transfections" on page 31.)

GETTING STARTED: Delve into the paper's supplemental information (*Nat Biotechnol*, 33:73-80, 2015). "We tried to be as detailed as possible because we want as many labs as possible to benefit from this approach," Liu says. The supplement shows how the group tweaked numerous variables to optimize Cas9 delivery. The resulting experiments should be relatively straightforward for experienced molecular biologists, he adds.

CONSIDERATIONS: The group also showed that the strategy can be used to deliver other large cargo, such as Cre recombinase and TALENs, and Liu's lab is trying additional proteins.

COST: Making your own Cas9 protein costs about $300 for starting materials. Each new guide RNA costs about $50 (for labor and supplies) if you already have the transcription and purification kits ($500).

BREAKING DOWN THE BULGE

COSMID identifies two new categories of potential off-target sites for CRISPR/Cas9

RESEARCHER: Gang Bao, professor of biomedical engineering, Georgia Institute of Technology

PROJECT: Gene-targeting strategies for treating single-gene disorders

PROBLEM: The pairing sequence of a guide RNA that is, for example, 20

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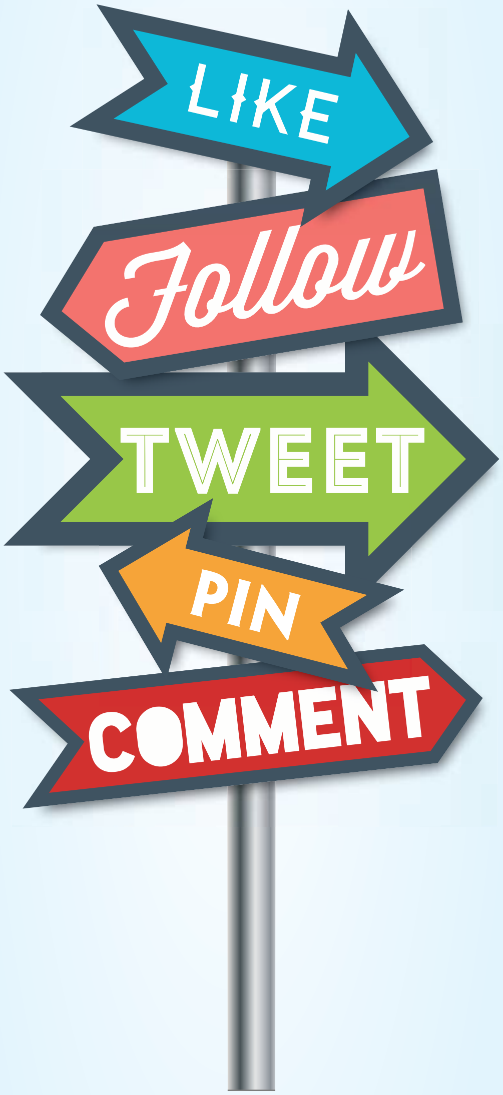

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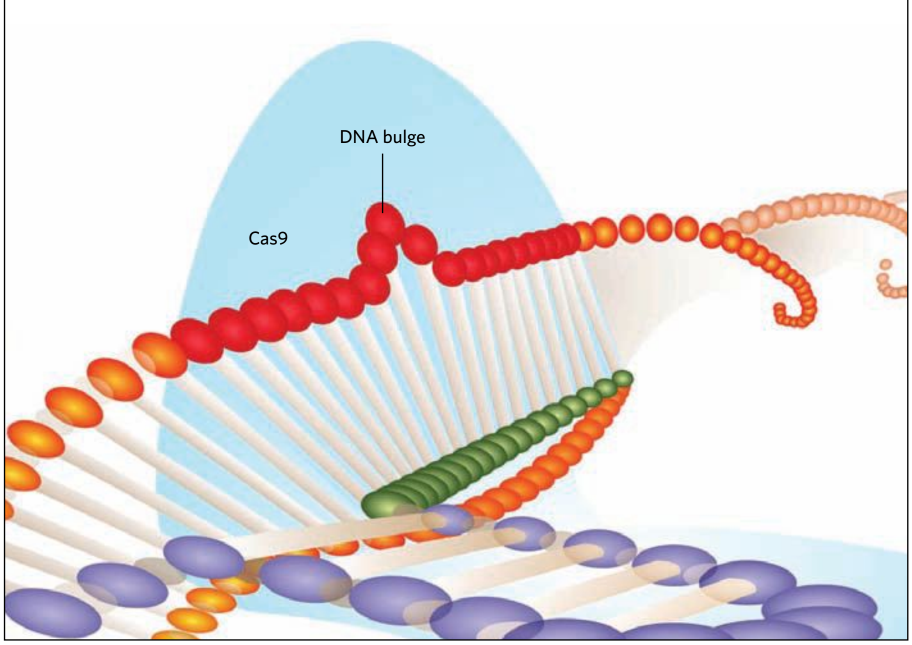

UNWELCOME EDITS: The Cas9 protein (light blue) and guide RNA (green and purple strands) can still bind to off-target stretches of DNA (orange-red), despite differences in length between the DNA and the guide pairing sequence, which produces bulges in the DNA.

bases long will probably match perfectly to its target sequence in the genome, but it can also bind to 20-base-long stretches with base mismatches, as well as to other off-target sites.

These days, numerous Web-based tools are available for pinpointing off-target sequences, but the algorithms miss two additional categories of nonspecific binding that occur despite differences in length between guide RNA and the DNA it binds. For example, if the RNA guide's pairing sequence is a base or two longer than the DNA but otherwise a perfect match, it may still bind to the DNA but in doing so produce an "RNA bulge." Conversely, if the DNA sequence is slightly longer than the guide's sequence, then it could create a "DNA bulge."

Indeed, last year Bao's group showed that off-target binding of the bulge variety can result in unwanted edits (*Nucleic Acids Res*, 42:7473-85, 2014). "With all those cases, we have a lot more to worry about in terms of specificity [of the guide for its intended target]," Bao says.

SOLUTION: Motivated to identify these potential off-target effects, the group launched COSMID, which, along with many other CRISPR tools, can help users design an RNA guide to minimize the potential of nonspecific cleavage by Cas9 (*Mol Ther Nucleic Acids*, 3:e214, 2014). If the user has already made a guide, he or she can use the tool to search for potential off-target spots in the genome.

GETTING STARTED: On COSMID's website (crispr.bme.gatech.edu), select the genome of interest from the list, then enter your guide-strand sequence and related parameters to screen for the best design. Or you can use the primer-design box to help validate the potential off-target sites.

CONSIDERATIONS: COSMID, as with all other CRISPR design tools, not only identifies potential off-target sites, it also ranks them according to the likelihood they will occur. "The ranking itself is not so accurate," Bao says, because many other factors play into these probabilities, so predicted off-target sequences would need to be validated experimentally.

Meantime, Vanderbilt's Mortlock suggests you weigh the predictions in the context of your specific project goals. For instance, if you want to remove a protein product from a cell line, you may not care too much about mutating another gene. On the other hand, if you are trying to connect a mutation with a specific phenotype, then checking for off-target effects is essential, he says.

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Riding Out Rejection

How to navigate the choppy waters of scientific publication

BY KATE YANDELL

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In July 2012, while attending the 12th International Coral Reef Symposium in Cairns, Australia, Emily Darling received a heart-wrenching e-mail: *Ecology Letters* had rejected her paper on coral life histories. She was shocked. The paper had already gone through one very positive round of reviews, and Darling, then in the final months of her PhD program at Simon Fraser University outside Vancouver, Canada, had resubmitted it with the requested changes. Just the day before, a senior colleague at a cocktail hour had revealed to her that he was one of the reviewers and that he had recommended that the journal publish her paper. Another reviewer had clearly disagreed.

"It felt like the worst place in the world that I ever could have got the rejection," says Darling, who received the e-mail in a conference room full of her peers. "I'm looking around at this amphitheater of people and saying, 'Who did it?' It sort of personalized the rejection experience."

But Darling's advisor, Isabelle Côté, wasn't about to take the rejection without a fight. The pair tracked down the paper's editor, who was also at the conference, and learned that the negative reviewer had been added in the second round because two first-round reviewers had been unavailable. Darling and Côté asked the editor if he would consider a letter rebutting the negative reviewer's criticisms, then tracked down the positive reviewer Darling had met, who agreed to send a letter vouching for the paper. A couple of months later, after the authors addressed some legitimate critiques from the positive reviewer and submitted a third draft, the paper was published (*Ecol Lett*, 15:1378-86, 2012).

Coping with publication rejection is a key skill for any academic. It can require emotional fortitude, an ability to profit from criticism, and, sometimes, negotiating skills. Many papers in the scientific literature were initially rejected, often sparking vigorous scientific discussions before their eventual publication, whether in the same journal or a different one.

"It does really take until you are a postdoc to start to be able to reflect more broadly on rejection and see the forest for the trees," says Darling, who is now a David H. Smith Conservation Research Fellow at the University of North Carolina at Chapel Hill. "You've done it enough by then that it's not a knife in the back every time a rejection comes in."

Here, *The Scientist* gathers researchers' strategies for navigating the peer-review process.

Take proper aim

One of the first decisions researchers have to make when preparing to publish a manuscript is where to submit. "I think it's important to know the type of paper that you have and who would be potentially interested in it," says cognitive neuroscientist Jane Wang, who is currently a postdoc at Northwestern University's Feinberg School of Medicine. "You don't want to waste your time going for journals that are not even going to take a [serious] look at it."

Jason McDermott, a computational biologist at the Pacific Northwest National Laboratory in Washington State, learned that lesson firsthand as a postdoc, when he and his mentor were convinced his paper was worthy of a top-tier journal. In the end, the paper was never published. "It got rejected [at least five] times and then it went off to die," says McDermott, who suspects it would have been published had they aimed their sights at a lower-impact publication.

For the most part, however, scientists have a good sense of where their papers are most likely to be accepted. Of more than 80,000 papers published in 923 biology journals from 2006 to 2008, 75 percent were published in the first journal they were submitted to, according to a 2012 study (*Science*, 338:1065-69). Lead author Vincent Calcagno conceived the work while he was dealing with a string of rejections of a paper from his PhD work at the University of Montpellier in France. Calcagno, now an evolutionary biologist and ecologist at the French Institute for Agricultural Research, says he was pleased to see that so many scientists reported acceptance by the first journal to which they submitted their research. "It made me more confident in the process . . . because rejection was not the rule."

For early-career scientists who are not yet sure where to submit, asking senior colleagues for suggestions is a good way to start. It may also be possible to gauge a journal's interest by providing a short summary of the paper before going to the trouble of a full submission. Some journals have formal presubmission processes. *Nature*, for instance, says that editors usually respond to presubmissions in two working days. In other cases, it's possible to more informally ask editors about their interest in a paper. Additionally, a couple of new companies now offer to do the work for you, sending out papers to independent reviewers before they are ever submitted to a journal. (See "A Helping Hand" on opposite page.)

Profit from criticism

Once you've selected the journal you're going to submit to, it's time to format the paper's figures and text to meet the journal's requirements. All journals have a set of submission standards. Check guidelines on length, structure, and technical term definitions, and read through past articles from the journal to get a sense of style and tone.

Once you submit, it becomes a waiting game. In some cases, the journal editors will decide not to send the paper out for review. This decision should be made within a few weeks. If your manuscript passes this first evaluation, the peer-review process can take anywhere from weeks to months. Many journals, including the Cell Press research journals, *PNAS*, and *Scientific Reports*, say that they try to respond to submissions within three weeks to a month. Others set more expansive limits or don't specify response times. The *Journal of Ecology*, for instance, promises to either respond to submissions within three months or explain why the process is delayed.

Such wait times may be an important factor to consider in deciding where to submit. Earlier this year, Santiago Salinas of the University of California, Santa Cruz, and Stephan Munch of the National Oceanic and Atmospheric Administration published a mathematical strategy to weigh citation numbers, acceptance rates, and average review times of 61 ecology journals (*PLOS ONE*, doi:10.1371/journal.pone.0115451, 2015). One could choose to maximize citations over a given time period, or to minimize number of resubmissions or total review time.

If the journal's long-awaited response is the dreaded rejection letter, take a deep breath: read through it to get a sense of its major points, then step away. "The emotion is high when you get a negative decision," says Lakshmi Goyal, editor of the journal *Cell Host and Microbe* and an editor at Cell Press since 2001. "[Later], go back and . . . look rationally at what is being asked and how it can be addressed."

While some reviews can be snide or dismissive, others offer well-reasoned advice. The trick for getting the most out of reviews, Wang says, is to assume the best of the reviewer. "I never go at it with the mind-set that they are against me," she says. "I always try to assume they are trying to help me."

Nikolaus Grigorieff, a structural biologist at the Howard Hughes Medical Institute's Janelia Research Campus in Ashburn, Virginia, points out that, like any type of written communication, manuscript reviews often do a poor job of portraying tone. A simple list of a paper's shortcomings can come across as extremely harsh, when the reviewer may have simply been in a rush. And even if a review is overtly scathing, it's still important to look beyond the nasty comments, says Grigorieff, who hosts an online repository of rejected papers, mainly from his own lab, and their reviews. "Sometimes reviewers are very disrespectful. You have to ignore that part and just extract the information that is there."

Indeed, while rejection is frustrating, it can ultimately benefit a paper. Calcagno's *Science* paper analyzing rejection showed that manuscripts that had been rejected from one or more journals before ultimately finding a home tended to be cited more than papers that sailed directly into the pages of that same journal. "Most papers can be much improved by more work on them," Calcagno says. "When we are rejected, we are forced to do that."

Also key to getting the most out of a review is to not approach comments too literally, Cell Press's Goyal advises. If a reviewer proposes an unrealistic new experiment, the author should try to understand why the reviewer is asking for the experiment and then try to find another way to answer the question. It is a mistake to dismiss criticisms outright, even if a reviewer is making inappropriate suggestions, Goyal says.

Darling agrees that taking time with reviewers' comments is worth it. "The worst thing you can ever do in my opinion is just resubmit the same paper to another journal

> **Most papers can be much improved by more work on them. When we are rejected, we are forced to do that.**
>
> —Vincent Calcagno, French Institute for Agricultural Research

without addressing the comments at all," she says. Editors' comments should also be taken seriously, she adds, as they often have years of experience in both science and publishing.

She recalls that the first paper she ever submitted was a very long manuscript attempting to analyze 20 years' worth of data on changes in coral ecosystems in Kenya. The editors of *Ecology* sent the paper out for review but then rejected it, telling her that it was far too long and that some of the paper's conclusions were unsupported. Darling went back and revised, ultimately publishing part of the work in *Conservation Letters* (3:122-30, 2010) and another bit a few years later in *Global Change Biology* (19:1930-40, 2013). "That rejection laid out the path of my PhD," she says. "It became a much stronger body of work because of that rejection."

Of course, reviewers can make mistakes. In some cases, reviewers simply missed a key point that was not well explained. "Oftentimes papers are rejected because of a misunderstanding," says Nikolai Slavov, a postdoc in quantitative cell biology at Harvard University. In such cases, it may be warranted to fight the rejection.

"You can't fight them all; that's not the point of peer review," says Darling. But sometimes "it's worth standing up for yourself."

Get perspective

Researchers should also recognize that no one journal is the key to career success. Slavov keeps a page on his blog of seminal papers that were initially rejected. In 1937, *Nature* rejected a paper from Hans Krebs on the now-eponymous Krebs cycle, which was published a couple months later in *Enzymologia* (4:148-56, 1937). (See "*Nature* Rejects Krebs's Paper," *The Scientist*, March 2010.) Lynn Margulis's paper on endosymbiont theory was rejected about 15 times before landing in the *Journal of Theoretical Biology* in 1967 (14:225-74). And both *Nature* and *Science* rejected Kary Mullis's paper on the polymerase chain reaction, which was ultimately published in *Methods in Enzymology* in 1987 (155:335-50).

More recently, David Botstein, now at Princeton University, Michael Eisen, now at the University of California, Berkeley, and their colleagues had their paper on cluster analysis, a widely used method for interpreting microarray data, rejected by *Science*. Botstein recalls phoning the editor to unsuccessfully appeal the rejection. "The only thing I remember telling her was that it was my thought that this would someday be a citation classic, and in this case I was right," Botstein says. The paper, which was eventually published in *PNAS* (95:14863-68, 1998), now has nearly 15,000 citations, according to Google Scholar.

"The important thing is that you get it published," Botstein advises. "If it's good and it's new and it's true and important, people will find it." ■

A HELPING HAND

In recent years, a new strategy for getting papers accepted has sprung up: the pre-peer review process. New companies now offer to send out papers for review by independent scientists before they have been submitted to a journal. Such early feedback can help researchers target the most appropriate journals, and the pre-reviews can be submitted alongside the manuscript to help expedite the journal review process.

Axios Review, which currently focuses on evolution and ecology papers, asks authors to send in each manuscript with a ranked list of four target journals where they would like to see it published. Reviewers selected by the company comment not only on the substance of the paper, but also on its suitability for each target journal. Taking these comments into account, an editor at the company decides where Axios will be willing to refer the paper. If the authors agree, Axios will then send a presubmission inquiry, including the reviews, to the journal. If the journal editors decide that the paper is of interest to them, the author may then submit it through normal channels. If the editors are not interested, Axios will refer the paper to another target journal.

Journals including *Molecular Ecology, Evolutionary Biology*, and *Ecology and Evolution* have published papers reviewed by Axios. *Ecology Letters* even mentions its willingness to consider Axios referrals on its author guidelines page. If Axios successfully places a paper at a target journal, the authors must pay $250. If the process does not result in publication, the authors owe nothing. Axios reviewers and editors, who are unpaid volunteers, are given discounts if they wish to put their own papers through the Axios review process.

For those outside the evolution and ecology fields, a second company, called Rubriq, also sends out papers for review and placement recommendations. Rubriq broadly reviews papers in the biomedical field and is now even expanding beyond the life sciences. Its service costs $650 and also includes a plagiarism check. Its reviewers are paid $100 for their efforts and fill out formalized reports for each paper, giving scores on a 10-point scale for each of three categories: novelty and interest, quality of research, and quality of presentation. Authors can revise their papers based on their Rubriq report and attach the report to their ultimate submissions.

The stated goal of these companies is to reduce time wasted on submitting to inappropriate journals. They also hope that some journals speed the decision to publish by referencing pre-reviews and sending the paper out to fewer or no additional reviewers.

Stirring the Pot

How to navigate the slings and arrows of conducting "controversial" research

BY ALICE DREGER

Three important insights I've gained from studying what has happened to scientists whose research has ticked off activists working within social justice movements: (1) If you want to get in trouble, study sex. (2) Anybody who tells you "all publicity is good publicity" has never been accused of genocide. (3) Death threats are rarely grammatically correct. I illustrate each of these lessons in my new book, *Galileo's Middle Finger: Heretics, Activists, and the Search for Justice in Science*.

First off, Mama was right: *Sex is gonna get you in trouble*. While it is true that you can also tick people off by making challenging scientific claims about alien abductions, chronic Lyme disease, or race—especially race—the surest route to having your reputation threatened is to contradict some deeply held belief about sex.

So, you could follow the leads of people like Craig Palmer and Randy Thornhill, who dared to suggest that rape may involve our evolved biology, or Michael Bailey, who promoted the idea that eroticism may play a role in transgenderism, or Elizabeth Loftus and Melvin Guyer, who challenged the idea of "recovered memory" of childhood sexual abuse.

As these people discovered, when your work gets labeled "controversial," you should expect that few of those criticizing you will actually read your publications; they're much more likely to go after you as a caricatured messenger than to engage in dialogue about your actual message.

This, then, takes us to the second point: *Anybody who tells you "all publicity is good publicity" has never been accused of genocide*. Being accused of genocide isn't one of those things for which you start a clippings file that you figure your grandchildren might someday like to have.

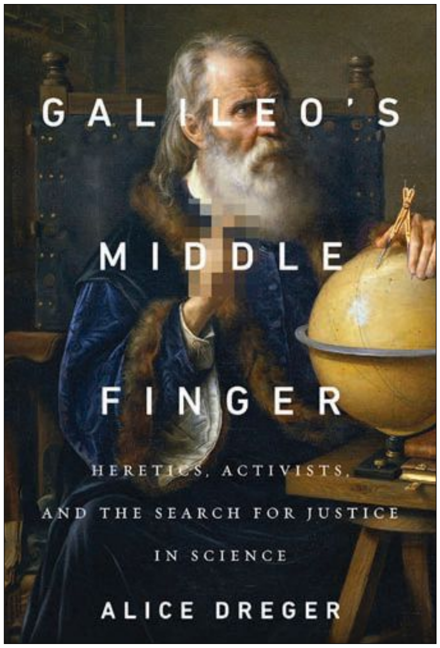
Just ask anthropologist Napoleon Chagnon, tried in absentia by the American Anthropological Association (AAA) because of allegations leveled in Patrick Tierney's 2000 book *Darkness in El Dorado*. Chagnon and geneticist James Neel were raked over the coals for two years in spite of the fact that Tierney's claims of unethical experimentation on South American natives were false and (as I show in my book) the leaders of the AAA's investigation team knew his book was "just a piece of sleaze."

Now, while the charge of genocide is usually reserved for those who are already renowned scientists, like Chagnon, the charge of being a eugenicist is readily available to the average scientist. For example, neuroscientist Charles Roselli's work on homosexual male sheep attracted charges of "gay eugenics"—ultimately leading to animal-rights and gay-rights proponent (and tennis champion) Martina Navratilova publicly condemning the research. You can't make this stuff up.

The good news: *Death threats are rarely grammatically correct*. Why is this good news? Because it suggests that the people who see themselves as your worst enemies—those who send violent threats—are typically not the well-educated, more reasonable people you have some chance of talking sense into.

And if, like Chagnon, you're lucky enough to have colleagues who bother to challenge falsehoods—or if you're super lucky, like Roselli, and have a university-relations expert named Jim Newman who helps you respond to 20,000 e-mails calling for your termination by disseminating facts about your work—then you have some hope.

Alternatively, you could try to avoid controversial work. The thing is, thanks to the Internet, just about anything you study could incite controversy. So instead, I'd suggest this: (1) The minute you're in hot water, create a plain-language FAQ about your work; (2) Don't believe what you read about yourself on the Internet; (3) Take comfort in being in truly excellent company among all the other eugenicists. I know I do. ■



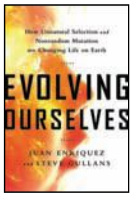
Penguin Press, March 2015

Alice Dreger is a historian of science and medicine and professor of clinical medical humanities and bioethics at the Feinberg School of Medicine, Northwestern University in Chicago. Read an excerpt of Galileo's Middle Finger *at www.the-scientist.com.*

Evolving Ourselves: How Unnatural Selection and Nonrandom Mutation Are Changing Life on Earth

Juan Enriquez and Steve Gullans

Current, March 2015



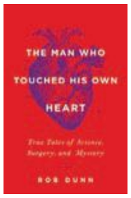
Evolution is too often referred to in the past tense. Author Juan Enriquez and erstwhile Harvard researcher Steve Gullans remind us, in *Evolving Ourselves*, that not only is evolution still churning away today, but humans have become major forces in the process by dint of our massive impact on the rest of the biosphere and on our own health and longevity. By diagnosing and accommodating individuals with severe food allergies, for example, we've enshrined a trait that was often fatal—and, therefore, rare—a mere three centuries ago.

Rather than bemoaning humanity's impact on natural selection, Enriquez and Gullans celebrate the potential to further alter our evolutionary course utilizing the modern marvels of biotechnology. Not coincidentally, the authors are also cofounders of Excel Venture Management, which invests in startups specializing in synthetic biology, big data, and new genetic technologies. Life's future is now firmly in our hands, they write, but only if we apply our increasing scientific knowledge with wisdom and foresight.

The Man Who Touched His Own Heart: True Tales of Science, Surgery, and Mystery

Rob Dunn

Little, Brown and Company, February 2015



Rob Dunn, the North Carolina State University biogeographer and popular science author, is back with *The Man Who Touched His Own Heart*. In his latest book, Dunn explores the history of humanity's centuries-long curiosity about this vital organ, from ancient civilizations that considered the heart the seat of the soul to researchers and clinicians of the modern era working on the fringes of experimental medicine to explore the mysteries of the circulatory system's workhorse.

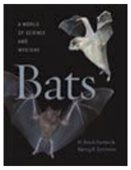
The book reaches its most dramatic heights as Dunn relates the story of his titular hero, Werner Forssmann. In 1929, the 25-year-old German physician sat at a bar one night and had an idea that would end up revolutionizing medicine. Forssmann hypothesized that it would be possible to send a catheter to the heart through a major vein or artery and obtain an image of the living organ in patients. His supervisors were dead set against the young clinician experimenting on humans, so Forssmann tricked a nurse into helping him perform the procedure on himself. He eventually obtained an X-ray of his own heart, an image that would win him a share of 1956's Nobel Prize in Physiology or Medicine, much to his own surprise.

With the engaging prose that has become his trademark, Dunn plumbs the depths of the heart, through the eyes of the ancients, early researchers, and contemporary scientists.

Bats: A World of Science and Mystery

M. Brock Fenton and Nancy B. Simmons

University of Chicago Press, January 2015



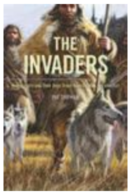
Blink, and you may miss almost a quarter of Earth's mammalian species. As they flit in and out of the shadows, bats live relatively secretive lives, but the roles they play in ecosystems that span the globe are hard to miss. From pollination and insect control to seed dispersal and soil improvement, more than 1,300 species of the flying mammals yield some of their secrets in *Bats: A World of Science and Mystery*, from bat researchers M. Brock Fenton and Nancy B. Simmons.

Alongside vivid photographs of bats flying, mating, and feeding, Fenton and Simmons explain the intricacies of the animals' ecology, behavior, and physiology. Above all, the book is a call to conserve bats, as encroachment and hunting threaten their habitats and populations. More than half of the world's bat species are in decline or already classified as endangered.

The Invaders: How Humans and Their Dogs Drove Neanderthals to Extinction

Pat Shipman

Belknap Press, March 2015

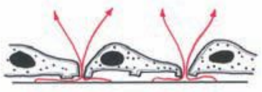


Europe 45,000 years ago was a battlefield of epic proportions. As bands of *Homo sapiens* arrived on the continent after departing their evolutionary birthplace in Africa, they encountered Neanderthals, a hominin species descended from groups that had migrated from Africa as much as a quarter of a million years earlier. In short order, Neanderthals disappeared from the landscape, while the more recent arrivals thrived. One of the keys to the newcomers' victory? Their canine companions.

The particulars of the story can only be conjectured, and ancient DNA evidence has suggested that we assimilated, rather than subjugated, Neanderthals. But in *The Invaders*, Penn State anthropologist Pat Shipman draws upon data from the field of invasion biology to build a case that modern humans were a major factor in the demise of our Neanderthal cousins, with a lot of help from our hunting partners, domesticated wolf-dogs. Our canines may have given us the much-needed edge in hunting down Ice Age mammals, which fueled our bodies and expanded our populations, Shipman argues. The book lays out a compelling argument not only for the importance of canine domestication in the rise of our species, but for how befriending dogs shaped our minds and mentalities as we advanced into the new age of *Homo sapiens* dominance.

—Bob Grant

Barrier Function of Endothelial Cells



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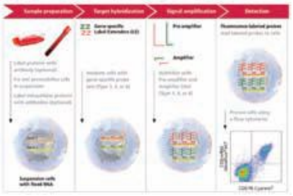

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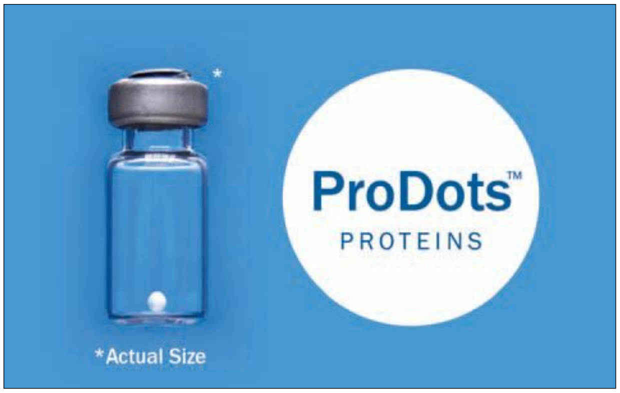

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Apiarium, 1625

BY KERRY GRENS

Around the turn of the 17th century, humans' narrow, mesoscale view of the world was undergoing a dramatic expansion. Not only was the telescope invented—allowing people for the first time to peer into space at distances we still find hard to fathom—but the microscope as well. And standing in the middle of all these technological advances was Galileo Galilei, remembered chiefly for his contributions to the former invention.

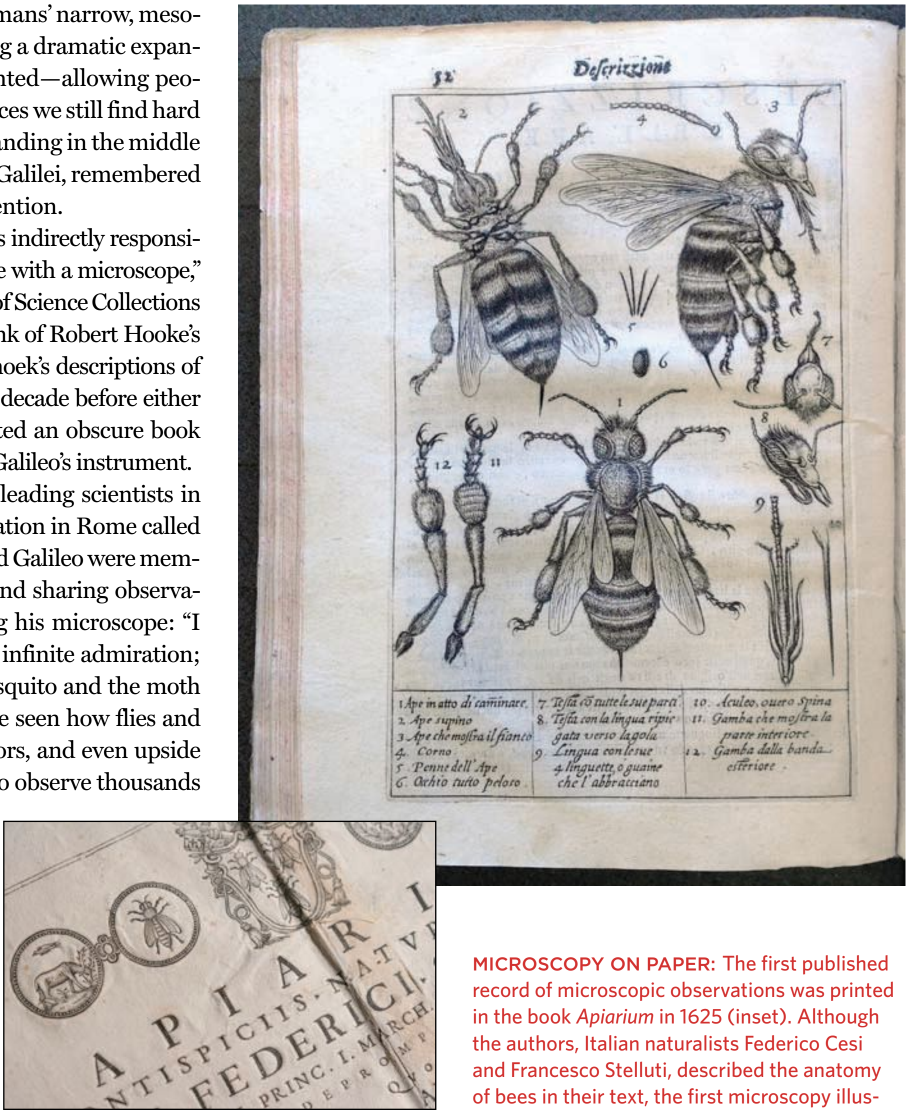
"Many people do not realize that Galileo was indirectly responsible for the first publication of observations made with a microscope," says Kerry Magruder, the curator of the History of Science Collections at the University of Oklahoma. People often think of Robert Hooke's famous sketch of a flea or Anton van Leeuwenhoek's descriptions of cells as the first microscopic publications, but a decade before either man was born, a pair of Italian scientists printed an obscure book describing what they saw down the eyepiece of Galileo's instrument.

Federico Cesi and Francesco Stelluti were leading scientists in their day. Cesi had founded a scientific organization in Rome called the Academy of the Lynxes, and both Stelluti and Galileo were members of the club, devoted to exploring nature and sharing observations. Galileo wrote to Cesi in 1624 describing his microscope: "I have contemplated a great many animals with infinite admiration; among them, the flea is most horrible, the mosquito and the moth are beautiful; and with great satisfaction I have seen how flies and other tiny creatures can walk attached to mirrors, and even upside down. But Your Excellency will have occasion to observe thousands and thousands of details, of which I request you to notify me of the most curious things."

Stelluti and Cesi used Galileo's compound microscope to view never-before-seen anatomical structures of bees, described in their 1625 text *Apiarium*—marking the first published microscopic discoveries. Seeking a powerful patron, the duo chose bees to honor Maffeo Barbarini, a cardinal and friend of Galileo whose family crest was a trio of bees, and who had been elected Pope Urban VIII in 1623. "The origins of classical microscopy are embedded in diplomacy," says Magruder.

Only six copies of *Apiarium* are known to exist, one of which is at the University of Oklahoma. The text will anchor an upcoming exhibit on Galileo at the university's libraries this year. Magruder says that one of the striking aspects of the book is that the authors used a tabular format to organize information, which offered a means of visually representing their understanding of nature.

Several years after the publication of *Apiarium*, Stelluti followed up with another book, *Persio*, in 1630, this time mingling literature and natural history. In the same year, Cesi died at the age of 45, and the Academy of the Lynxes soon dissolved without his leadership.



MICROSCOPY ON PAPER: The first published record of microscopic observations was printed in the book *Apiarium* in 1625 (inset). Although the authors, Italian naturalists Federico Cesi and Francesco Stelluti, described the anatomy of bees in their text, the first microscopy illustrations were published five years later in Stelluti's book, *Persio*. The arrangement of the bees in his illustration (above) echo the trio of bees in the family crest of Maffeo Barbarini, also known as Pope Urban VIII. The scientists' tribute to Barbarini is evident also in their descriptions of the bees as Urbanas (domestic) and Barbaras (rural). According to a 1970 translation of *Apiarium* by Clara Sue Kidwell, Cesi and Stelluti wrote: "You have distinguished Urban bees by their customs, virtue, worthiness and barbarian bees by their swarms, purity, and harmony in singing."

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Magruder points out that "it's remarkable that the lynx emblem, which was chosen because of the lynx's reputed vision at night, turned out to be a metaphor immediately extended by Galileo's microscope and telescope." ■

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